Equine Veterinary Journal

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/evj.13085

Effects of vatinoxan on cardiorespiratory function and gastrointestinal motility during constant-rate medetomidine infusion in standing horses

H. TAPIO[†]*, M. R. RAEKALLIO[†], A. MYKKÄNEN[†], S. MÄNNIKKÖ[‡], M. SCHEININ[§,¶], R. C. BENNETT[†] and O. VAINIO[†]

[†]Faculty of Veterinary Medicine, Department of Equine and Small Animal Medicine, University of Helsinki, Helsinki, Finland
[‡]4Pharma Ltd., Turku, Finland
[§]Institute of Biomedicine, University of Turku, Turku, Finland
[¶]Unit of Clinical Pharmacology, Turku University Hospital, Turku, Finland.

*Correspondence email: heidi.tapio@helsinki.fi; Received: 21.06.18; Accepted: 17.02.19

Summary

Background: Medetomidine suppresses cardiovascular function and reduces gastrointestinal motility in horses mainly through peripheral α_2-adrenoceptors. Vatinoxan, a peripheral α_2-antagonist, has been shown experimentally to alleviate the adverse effects of some α_2-agonists in horses. However, vatinoxan has not been investigated during constant-rate infusion (CRI) of medetomidine in standing horses.
Objectives: To evaluate effects of vatinoxan on cardiovascular function, gastrointestinal motility and on sedation level during CRI of medetomidine.
Study design: Experimental, randomised, blinded, cross-over study.
Methods: Six healthy horses were given medetomidine hydrochloride, 7 μg/kg i.v., without (MED) and with (MED+V) vatinoxan hydrochloride, 140 μg/kg i.v., followed by CRI of medetomidine at 3.5 μg/kg/h for 60 min. Cardiorespiratory variables were recorded and borborygmi and sedation levels were scored for 120 min. Plasma drug concentrations were measured. The data were analysed using repeated measures ANCOVA and paired *t*-tests as appropriate.
Results: Initially heart rate (HR) was significantly lower and mean arterial blood pressure (MAP) significantly higher with MED compared with MED+V. For example at 10 min HR (mean ± s.d.) was 26 ± 2 and 31 ± 5 beats/minute ($P = 0.04$) and MAP 129 ± 15 and 103 ± 13 mmHg ($P<0.001$) respectively. At 10 min, cardiac index was lower ($P = 0.02$) and systemic vascular resistance higher ($P = 0.001$) with MED than with MED+V. Borborygmi were reduced after MED; this effect was attenuated by vatinoxan ($P<0.001$). All horses were sedated with medetomidine, but the mean sedation scores were reduced with MED+V until 20 min (6.8 ± 0.8 and 4.5 ± 1.5 with MED and MED+V, respectively, at 10 min, $P = 0.001$). Plasma concentration of dexmedetomidine was significantly lower in the presence of vatinoxan ($P = 0.01$).
Main limitations: Experimental study with healthy, unstimulated animals.
Conclusions: Vatinoxan administered i.v. with a loading dose of medetomidine improved cardiovascular function and gastrointestinal motility during medetomidine CRI in healthy horses. Sedation was slightly yet significantly reduced during the first 20 min..

The Summary is available in Portuguese – see Supporting Information

Keywords: horse; sedation; medetomidine; vatinoxan; MK-467; α_2-antagonist

Introduction

The α_2-adrenoceptor agonists (α_2-agonists) administered by constant-rate infusions (CRI) are commonly used to provide sedation for standing surgical and diagnostic procedures. Medetomidine, with its pharmacologically active enantiomer dexmedetomidine, is the most selective α_2-agonist in clinical use and provides sedation and analgesia through centrally mediated effects [1]. In horses, its pharmacokinetic profile, with a short half-life, makes it attractive for use in CRI [2,3], although it is registered for use only in small animals. Infusion rates for stable sedation with medetomidine and dexmedetomidine in horses have been described [2–4]. However, medetomidine shares the various adverse peripheral effects of all α_2-agonists. Most notably, medetomidine causes vasoconstriction, leading initially to hypertension and baroreceptor-mediated reflex bradycardia [5], later followed by a centrally mediated hypotension [4]. At equipotent doses, medetomidine-induced bradycardia is shorter in duration than that induced by detomidine [6]. Additionally, medetomidine decreases mixed venous oxygen tension [4]. Dexmedetomidine also decreases borborygmi and increases blood glucose concentrations in horses [7].

Vatinoxan (previously MK-467 or L-659,066) is a peripherally acting α_2-adrenoceptor antagonist. In rats and marmosets, vatinoxan crosses the blood–brain barrier minimally such that its concentration in the brain remains far below that present in blood [8]. Thus, vatinoxan may reduce the peripheral adverse effects of α_2-agonists without interfering with their centrally mediated sedative or analgesic effects. In previous equine studies, vatinoxan prevented the adverse cardiovascular effects of simultaneously administered single doses of the α_2-agonists medetomidine [9], detomidine [10] and romifidine [11]. Although vatinoxan increased the volume of distribution and clearance of detomidine [10] and romifidine [11] in horses, it did not have clinically relevant effects on the degree of sedation. Furthermore, vatinoxan improved gastrointestinal motility in horses when administered with single doses of detomidine [10,12] and romifidine [11].

The objective of this study was to evaluate the impact of vatinoxan on cardiorespiratory and gastrointestinal parameters during a CRI of medetomidine in standing, healthy horses. In addition, the effects of vatinoxan on sedation and plasma concentrations of the enantiomers of medetomidine (dexmedetomidine and levomedetomidine) were investigated. We hypothesised that vatinoxan would alleviate the cardiovascular

and gastrointestinal depression caused by medetomidine but would not lead to clinically relevant loss of sedation.

Materials and methods

Horses

Six horses (4 Standardbreds and 2 Warmbloods; 3 mares and 3 geldings) with a median age of 10 years (range, 5–19 years) and median body weight of 513 kg (range, 460–557 kg) were used in the study. The horses were considered healthy on the basis of clinical examination and results of complete blood count and serum biochemistry. Horses were fed hay and had free access to water, except during the experiment. Feed, but not water, was withheld for 2 h before and after the experiment.

Instrumentation

Instrumentation was performed with horses unmedicated and restrained in stocks. First, vascular catheters were aseptically placed with 2 mL of 2% mepivacaine (Scandicain)[a] injected subcutaneously for local anaesthesia. A central venous catheter (Cavafix Certo)[b] was placed into the cranial vena cava via the distal part of the left jugular vein. The correct location of the central venous catheter was verified by characteristic pressure wave forms on the monitor screen (S/5 compact Critical Care Monitor)[c]. The proximal part of the same left jugular vein was then used for a standard intravenous catheter (Intraflon)[d]. An arterial catheter (BD Arterial cannula)[e] was placed into the previously subcutaneously relocated [13] right common carotid artery. Finally, a lithium sensor (LiDCO Plus)[f] was connected to the arterial catheter for cardiac output (Qt) determination by the lithium dilution method (LiDCO Plus)[f] [14]. Lithium was prepared as previously described by adding 1.875 mL of 8 mol/L lithium chloride to 100 mL of saline (0.9%) solution to obtain a concentration of 150 mmol/L [12]. Standard values of 6.2 mmol/L (10 g/dL) (haemoglobin) and 140 mmol/L (sodium) were entered. The calculation of Qt was later performed with the true values of haemoglobin and sodium at each time point as measured from the arterial blood samples.

Treatments and data collection

Prior to drug administration, baseline values for all measurements were determined. Heart rate and respiratory rate were obtained by auscultation, and Qt, systolic (SAP), mean (MAP) and diastolic (DAP) arterial blood pressure and central venous pressure (CVP) were recorded. Arterial and venous blood was anaerobically collected into heparinised syringes (Pico50)[g] and stored on ice. Blood gas analysis (ABL800 Flex)[g] was performed within 10 min of sample collection. Values were temperature-corrected on the basis of concurrently recorded rectal temperature. An investigator (M.R.R., R.C.B.) blinded to the treatment assignment assessed sedation according to a predefined sedation scoring system [15] (Supplementary Item 1). The same investigator then auscultated gastrointestinal borborygmi and scored it following a previously described scale [16] (Supplementary Item 2).

Each horse received two treatments in a randomised cross-over design, separated by at least 7 days. Horses were administered medetomidine hydrochloride[h] (Dorbene) (7 μg/kg, i.v.) over 15 s through the jugular catheter at time 0 without (MED) or with (MED+V) vatinoxan hydrochloride[i] (140 μg/kg i.v.). The drugs were diluted in saline to achieve a total injection volume of 10 mL. Five min following the bolus, a CRI of medetomidine was initiated at a rate of 3.5 μg/kg/h and continued for 60 min with an infusion pump (Perfusor)[b].

Heart rate, respiratory rate, SAP, DAP, MAP and CVP were recorded at 5, 10, 20, 30, 45, 60, 90 and 120 min. From 10 min onwards, at the same time points, the Qt and blood gas values from arterial and venous blood samples were measured and sedation and gastrointestinal borborygmi were scored by the same blinded investigator.

In addition, arterial blood samples (10 mL) were collected into EDTA-containing tubes at 10, 30, 60, 90 and 120 min and stored at room temperature (20°C). Plasma was separated by centrifugation at 400 ***g*** for 10 min ≤6 h after sample collection and stored at −20°C until analysed for drug concentrations.

The following calculations were performed retrospectively: cardiac index (CI), stroke volume index (SVI), systemic vascular resistance index (SVRI), arterial oxygen content (Cao_2), venous oxygen content (Cvo_2), left ventricular workload (LVW), oxygen delivery index (DO_2I), oxygen consumption index (VO_2I) and oxygen extraction (O_2ER) [17–19] (Supplementary Item 3). Pharmacokinetic parameters were calculated using commercially available software (Phoenix WinNonlin)[j] using non-compartmental analysis with the following settings: linear trapezoidal linear/log interpolation and uniform weighting for all analysed drugs, time range slope and dose type i.v. infusion for dex- and levomedetomidine, and best fit slope and dose type i.v. bolus for vatinoxan.

Analysis of plasma drug concentrations

The concentrations of drugs in plasma were determined with high-performance liquid chromatography-tandem mass spectrometry in separate aliquots that were processed under specific conditions for the two drugs. The concentrations were determined after solid-phase extraction with well extraction plates (Sep-Pak tC18)[k] with racemic d_3-medetomidine (Toronto Research Chemicals)[l] and RS-79948 (Tocris Biosciences)[m] as the respective internal standards. For dexmedetomidine and levomedetomidine analysis, chiral separation with an α1-acid glycoprotein (AGP) column (Chiralpak)[n] was performed and 10 mmol/L ammonium acetate (pH 4.5) and acetonitrile containing 0.1% formic acid were used as solvents. For vatinoxan, reversed-phase separation with a 2.1 × 150 mm, 3.5 μm C_{18} column (SunFire)[k] and a gradient solvent system (0.1% formic acid in water and acetonitrile) was used. Quantitative detection was performed in multi-reaction monitoring mode with a triple quadrupole mass spectrometer (4000 QTrap)[o]. For dexmedetomidine and levomedetomidine and for d_3-medetomidine, the respective precursor ions (m/z) were 201.2 and 204.2. The fragment ions (m/z) monitored and used for quantitation were 95.1 for dexmedetomidine and levomedetomidine and 98.05 for d_3-medetomidine. For vatinoxan and RS-79948, the respective precursor ions (m/z) scanned were 419.3 and 365.3. The fragment ions monitored and used for quantitation were 200.1 for vatinoxan and 190.2 for RS-79948. The chromatograms were then processed using MDS Sciex[o] software. The linear concentration range for the assay of dexmedetomidine and levomedetomidine was from 0.100 to 10.0 ng/mL. The inter-assay accuracy of the quality control samples (at three different concentration levels, 0.225, 1.0 and 8.0 ng/mL) ranged from 101.2 to 108.5% for dexmedetomidine and from 99.4 to 108.4% for levomedetomidine. The linear concentration range of the assay of vatinoxan was from 25 to 460 ng/mL. The accuracy of the quality control samples (at three different concentration levels, 70, 250 and 380 ng/mL) ranged from 102.5 to 113.6%.

Data analysis

All statistical analyses were performed using commercially available software (SAS[p] and SPSS[q]). The sample size was chosen based on practical considerations and previous evidence from comparable studies [10,12] indicating that to detect clinically relevant differences in the investigated parameters a sample size of six horses would be sufficient.

Change from baseline was used as the response with all variables, excluding plasma drug concentration data. The differences between treatments in change from baseline values were assessed with repeated measures analysis of covariance models. The model consisted of a baseline covariate, the main effects of treatment, period, sequence and time point of measurement and two-way interactions of period*time point, sequence*time point and treatment*time point as fixed effects, and main effect of horse and two-way interaction terms of period*horse and time point*horse as random effects. No carry-over effect was expected to arise in the study and was therefore not tested. The normality assumptions were checked for all responses with Kolmogorov–Smirnov tests. For SVI, DO_2I and VO_2I, logarithmic transformation was computed to normalise the distributions. For SAP, a square transformation and for CI an inverse transformation was used. With all transformed change variables, the model was fitted for changes in the transformed response. From the fitted models, estimates of treatment effect were calculated over time and by time point using model

contrasts. In addition, estimates for the within treatment changes were computed similarly from the same model.

For plasma concentration values and areas under the time-plasma concentration curves, normality was investigated with the Shapiro–Wilk test. The data were normally distributed and paired *t*-tests were applied.

P≤0.05 were considered statistically significant.

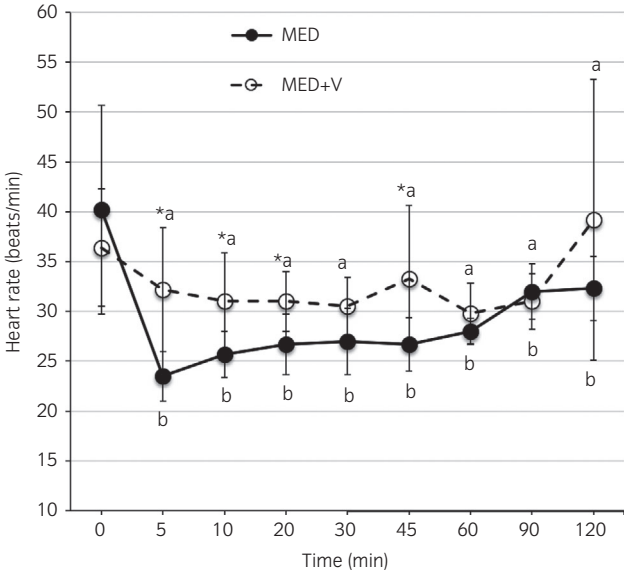

Fig 1: Mean (±s.d.) heart rate of 6 horses after administration of medetomidine hydrochloride 7 μg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 μg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 μg/kg/h administered from 5 to 65 min. Significant differences (P≤0.05) are labelled as within (a) MED and (b) MED+V with respect to baseline and as (*) between the treatments at given time points.

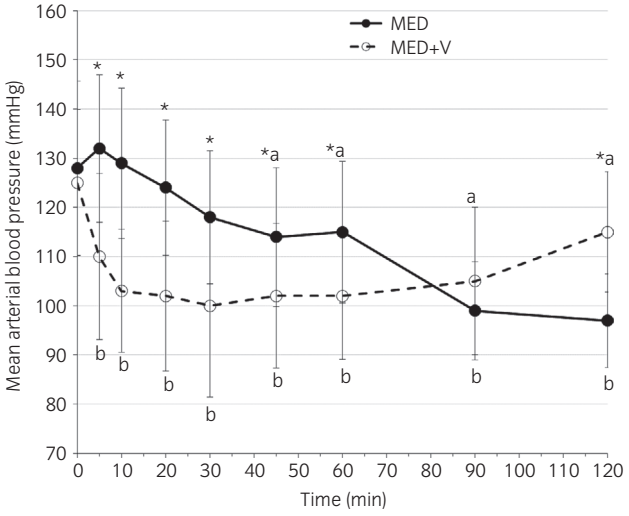

Fig 2: Mean (±s.d.) arterial blood pressure of 6 horses after administration of medetomidine hydrochloride 7 μg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 μg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 μg/kg/h administered from 5 to 65 min. Significant differences (P≤0.05) are labelled as within (a) MED and (b) MED+V with respect to baseline and as (*) between the treatments at given time points.

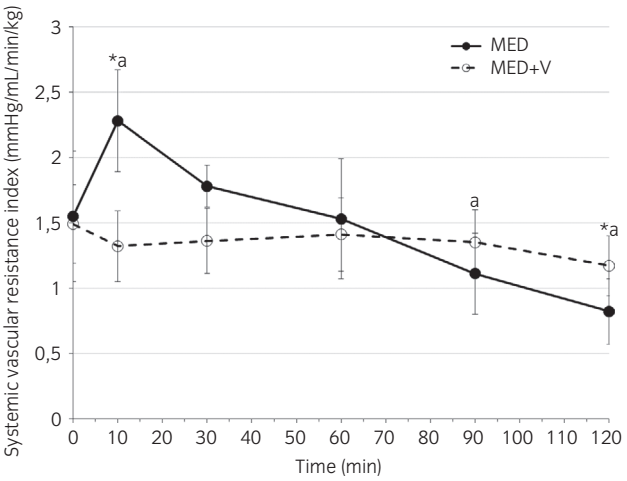

Fig 3: Mean (±s.d.) systemic vascular resistance index of 6 horses after administration of medetomidine hydrochloride 7 μg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 μg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 μg/kg/h administered from 5 to 65 min. Significant differences (P≤0.05) are labelled as within (a) MED with respect to baseline and as (*) between the treatments at given time points.

Results

Cardiovascular effects

Heart rate was overall significantly lower with MED than with MED+V; the lowest HR was 24 ± 3 beats/minute and 30 ± 3 beats/minute with MED and MED+V, respectively (Fig 1). With MED+V, MAP immediately decreased and was significantly lower than with MED until 60 min, with a nadir of 100 ± 19 mmHg at 30 min (Fig 2). MAP remained unchanged from baseline with MED until 45 min, after which MAP significantly decreased, with a nadir of 97 ± 10 mmHg at 120 min (Fig 2). Similar changes were observed in SAP and DAP (Table 1). A significant increase in CVP was observed with MED until 20 min, after which CVP remained at baseline levels, whereas a significant decrease in CVP was observed with MED+V at 30 min, lasting until 90 min.

With MED, the CI decreased and SVRI increased immediately and were significantly different from MED+V at 10 min (Table 1 and Fig 3). After cessation of the CRI, there was a significant decrease in SVRI and increase in SVI with MED (Table 1 and Fig 3). In comparison, no significant changes in CI, SVRI and SVI were detected with MED+V. Left ventricular workload significantly decreased after medetomidine administration, without significant differences between the treatments (Table 1).

Respiratory rate and arterial and venous blood gas analysis

Respiratory rate, PaO_2, PvO_2 and DO_2I significantly decreased, and O_2ER and CO_2 tensions in arterial and venous blood significantly increased over time with both treatments (data for 5 and 45 min and for CO_2 tension are not shown) (Table 2). However, significant differences were detected only in PvO_2 and in O_2ER, in which the changes from baseline were significantly greater with MED than with MED+V. No consistent changes in VO_2I were detected (Table 2). Arterial glucose concentrations did not change from baseline with MED+V whereas they increased significantly with MED, with the highest mean value of 9.6 ± 2.4 mmol/L (Table 2).

Borborygmi and sedation scores

While medetomidine significantly decreased the borborygmi score, the decrease was overall significantly smaller and briefer with MED+V than with MED (Table 1). The sedation score increased significantly from baseline in all horses within 10 min after administration of medetomidine; scores were increased from 0.7 ± 0.5 to 6.8 ± 0.8 and from 0.5 ± 0.6 to 4.5 ± 1.5 with MED and MED+V respectively. Sedation scores returned to

TABLE 1: Cardiovascular measurements, borborygmi and sedation scores (mean ± s.d.) of 6 horses after administration of medetomidine hydrochloride 7 μg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 μg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 μg/kg/h administered from 5 to 65 min

Variable	Treatment	Baseline	5 min	10 min	20 min	30 min	45 min	60 min	90 min	120 min
SAP (mmHg)	MED	161 ± 23.3	159 ± 16.5	159 ± 24.6	154 ± 17.7	146 ± 17.2†	141 ± 15.4†	134 ± 14.4†	123 ± 10.2†	123 ± 8.6†
	MED+V	166 ± 15.3	130 ± 20†*	127 ± 14.5†*	130 ± 18.3†*	125 ± 22.3†*	128 ± 17.1†*	124 ± 16.6†	128 ± 16.5†	148 ± 13.8†
DAP (mmHg)	MED	104 ± 12.7	116 ± 14.8†	114 ± 13.5	106 ± 12.3	102 ± 11	100 ± 9.4	99 ± 17.6	84 ± 8.3†	81 ± 8.0†
	MED+V	97 ± 15.2	93 ± 17.6*	89 ± 10*	89 ± 15.1*	85 ± 15.5†*	82 ± 15.6†*	86 ± 11.8	89 ± 11.5	93 ± 12.8
Mean CVP (mmHg)	MED	11.0 ± 3.9	15.0 ± 3†	13.7 ± 3†	12.3 ± 2.4	11.5 ± 3.5	11.0 ± 2.4	9.7 ± 1.6	9.5 ± 4	8.2 ± 4.2†
	MED+V	11.5 ± 3.3	9.2 ± 2.4*	9.7 ± 2.7*	9.3 ± 3.5*	7.2 ± 3.7†*	8.2 ± 2.6†*	8.0 ± 4.2†	8.8 ± 2.8†	11.0 ± 3.7
Cardiac index (mL/min/kg)	MED	80.8 ± 22.1	–	52.8 ± 6.9†	–	61.4 ± 3.9	–	72.4 ± 14.6	86.1 ± 26.3	120 ± 45.4†
	MED+V	77.0 ± 14.6	–	70.2 ± 12.6*	–	69.3 ± 9.9	–	67.7 ± 8.1	73.1 ± 18.1	92.9 ± 25.3
Stroke volume index (mL/kg)	MED	2.0 ± 0.5	–	2.1 ± 0.4	–	2.2 ± 0.2	–	2.6 ± 0.5†	2.6 ± 0.8†	3.7 ± 1.4†
	MED+V	2.2 ± 0.5	–	2.4 ± 0.5	–	2.3 ± 0.4	–	2.3 ± 0.3	2.3 ± 0.4	2.4 ± 0.4*
Left ventricular workload (kg m)	MED	72.2 ± 25.7	–	47.5 ± 7.0†	–	52.3 ± 10.9	–	57.1 ± 10	58.2 ± 14.1	79.8 ± 27.6
	MED+V	64.9 ± 14.7	–	47.3 ± 7.3†	–	48.6 ± 14.3†	–	47.4 ± 7.4†	54.4 ± 20.1†	74.6 ± 26.1
Borborygmi score	MED	4.9 ± 1.5	–	1.0 ± 0.8†	0.3 ± 0.3†	0.3 ± 0.4†	0.6 ± 0.5†	0.3 ± 0.4†	0.6 ± 0.6†	1.4 ± 0.7†
	MED+V	5.1 ± 2.0	–	4.3 ± 2.5*	3.4 ± 1.6†*	2.3 ± 1.2†*	2.6 ± 1.2†*	2.3 ± 0.9†*	5.3 ± 1.6*	4.5 ± 2.0*
Sedation score	MED	0.7 ± 0.5	–	6.8 ± 0.8†	6.8 ± 0.4†	6.5 ± 0.6†	6.3 ± 0.8†	5.2 ± 1.7†	2.8 ± 2.3†	1.5 ± 1.1
	MED+V	0.5 ± 0.6	–	4.5 ± 1.5†*	5.2 ± 1.3†*	5.5 ± 1.1†	5.3 ± 1.2†	4.7 ± 0.8†	2.0 ± 1.1†	1.2 ± 0.4

SAP, systolic arterial blood pressure; DAP, diastolic arterial blood pressure; CVP, central venous pressure. Significant differences (P≤0.05) are labelled as (†) within treatment with respect to baseline at given time point and (*) between the treatments in change from baseline at given time point.

TABLE 2: Respiratory rate, arterial and venous blood gas data and calculated variables derived from it, and plasma glucose concentrations (mean ± s.d.) of the same 6 horses as in Table 1

Variable	Treatment	Baseline	10 min	20 min	30 min	60 min	90 min	120 min
Respiratory rate (breaths/min)	MED	20 ± 9	12 ± 5†	10 ± 2†	12 ± 5†	8 ± 2†	8 ± 1†	8 ± 3†
	MED+V	15 ± 4	8 ± 1†*	9 ± 2†	7 ± 2†*	7 ± 2†	6 ± 2†	7 ± 3†
PaO_2 (mmHg)	MED	99.6 ± 3.6	93.5 ± 6.0†	–	95.2 ± 4.3†	98.7 ± 3.6	101.8 ± 6.8	105.5 ± 5.2†
	MED+V	98.8 ± 8.5	91.8 ± 3.3†	–	98.0 ± 4.3	99.1 ± 3.6	104.3 ± 3.4†	100.1 ± 3.6*
PvO_2 (mmHg)	MED	36.3 ± 6.3	26.2 ± 2.1†	–	28.3 ± 2.0†	29.2 ± 1.8†	31.1 ± 3†	30.0 ± 3.1†
	MED+V	33.8 ± 3.6	32.6 ± 1.9†*	–	31.8 ± 3.1†*	31.1 ± 3.1†	31.0 ± 2.8†	35.9 ± 4.1*
DO_2 index (mL/min/kg)	MED	14.9 ± 5.2	7.9 ± 1.1†	–	8.9 ± 1.3†	9.9 ± 2.2†	13.5 ± 6.4	19.7 ± 8.1†
	MED+V	13.7 ± 3.1	10.8 ± 1.4	–	10.3 ± 2.9†	9.6 ± 1.7†	10.0 ± 2.4†	16.1 ± 4.7
VO_2 index (mL/min/kg)	MED	3.3 ± 1.0	3.3 ± 0.3	–	3.2 ± 0.4	3.5 ± 1.0	4.0 ± 1.1	6.9 ± 3.7†
	MED+V	3.8 ± 1.2	3.0 ± 0.4	–	3.1 ± 0.7	3.0 ± 0.5	3.3 ± 0.8	3.7 ± 1.1*
O_2ER %	MED	23.9 ± 8.3	41.4 ± 5.2†	–	35.9 ± 5.3†	34.6 ± 3.9†	30.7 ± 5.2†	34.5 ± 6.4†
	MED+V	27.9 ± 6.9	28.2 ± 3.7*	–	30.4 ± 6.2†*	31.9 ± 6.0†	32.8 ± 6.1†	23.9 ± 7.4*
Plasma glucose concentration (mmol/L)	MED	5.5 ± 0.6	5.4 ± 1.5	–	7.8 ± 1.5†	9.4 ± 2.6†	9.6 ± 2.4†	8.9 ± 2.1†
	MED+V	5.7 ± 0.5	5.7 ± 0.5	–	6.5 ± 1.2	6.9 ± 1.5*	7.1 ± 1.6*	7.0 ± 1.3

PaO_2, arterial oxygen tension; PvO_2, central venous oxygen tension; DO_2 index, oxygen delivery index; VO_2 index, oxygen consumption index; O_2ER%, oxygen extraction ratio. Significant differences (P≤0.05) are labelled as (†) within treatment with respect to baseline at given time point and (*) between the treatments in change from baseline at given time point.

pre-treatment levels by 120 min after drug administration (Table 1). The increase in sedation score was significantly larger with MED than with MED+V at 10 and 20 min.

Pharmacokinetic results

The areas under the time-plasma concentration curves of dexmedetomidine and levomedetomidine were significantly smaller in the presence of vatinoxan, thus indicating lower plasma exposure to dexmedetomidine (Table 3, Fig 4). Indeed, the concentrations of dexmedetomidine in plasma during the CRI of medetomidine were also significantly lower with MED+V than with MED (Fig 4).

TABLE 3: Pharmacokinetic parameters (mean±SD) for dexmedetomidine, levomedetomidine and vatinoxan of the same 6 horses as in Table 1

Drug	Treatment	$AUC_{0-120\ min}$ (min ng/mL)	$AUC_{60-120\ min}$ (min ng/mL)	$t_{½}$ (min)
Dexmedetomidine	MED	72.0 ± 18.0	17.3 ± 5.9	19 ± 5[a]
	MED+V	58.3 ± 17.2*	15.3 ± 6*	20 ± 4[a]
Levomedetomidine	MED	29.3 ± 11.7	9.4 ± 3.5	20 ± 3[a]
	MED+V	24.7 ± 11.7*	8.4 ± 3.5	23 ± 2[a]
Vatinoxan	MED+V	8950 ± 2851	–	75 ± 28

AUC, area under time-concentration curve; $t_{½}$, half-life of the drug.
[a]These $t_{½}$ estimates are uncertain because the calculations were based on only three data points for each animal.
*Significant difference between MED and MED+V (P≤0.05).

Discussion

The results of this study support the hypothesis that a single i.v. bolus of vatinoxan prevents the cardiovascular and gastrointestinal effects induced by a loading dose and CRI of medetomidine without major effects on

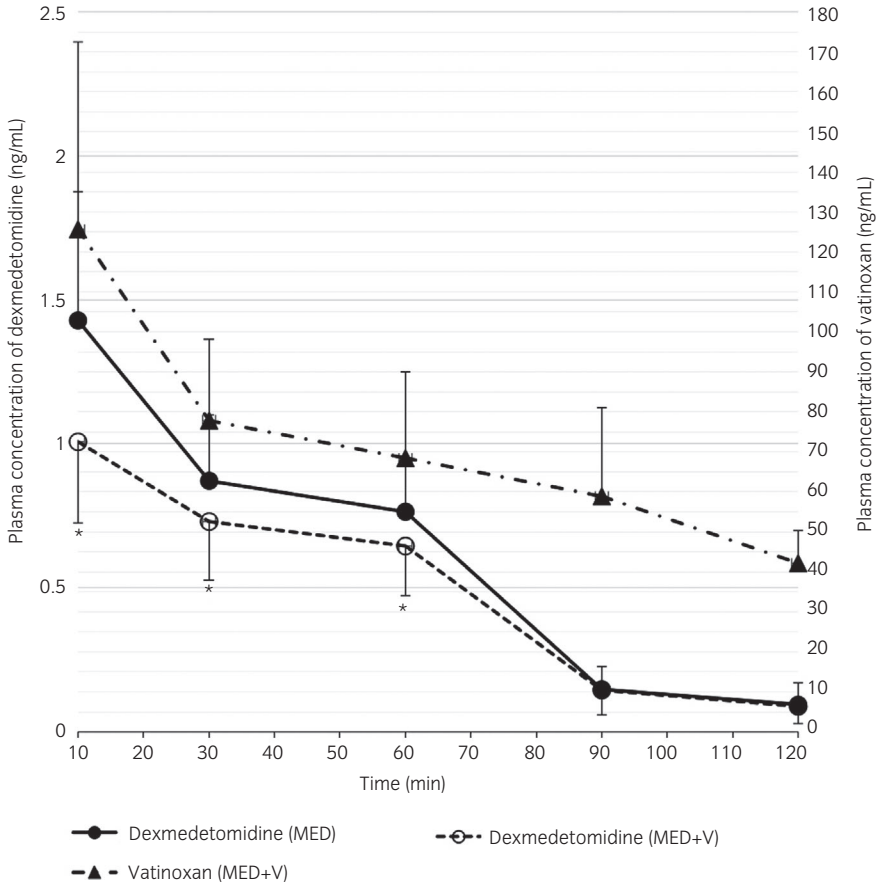

Fig 4: Mean (±s.d.) plasma concentrations of dexmedetomidine and vatinoxan of 6 horses after administration of medetomidine hydrochloride 7 μg/kg i.v. alone (MED) or with vatinoxan hydrochloride 140 μg/kg i.v. (MED+V) at 0 min and constant-rate infusion of medetomidine 3.5 μg/kg/h administered from 5 to 65 min. Significant differences ($P \leq 0.05$) are labelled as (*) between the treatments at given time points.

sedation. As expected, medetomidine, when administered alone, induced immediate vasoconstriction (observed as increased MAP and SVRI), even if MAP remained within clinically acceptable limits. From 60 min onwards MAP and SVRI decreased with MED, likely due to prevailing central sympatholytic effect of medetomidine [4]. In the presence of vatinoxan, MAP decreased but remained within the reference range of standing horses (94–140 mmHg) [20]. Furthermore, the calculated SVRI (describing peripheral vasoconstriction) remained stable with MED+V throughout the study period. The observations likely reflect the medetomidine-induced vasoconstriction that vatinoxan alleviated, probably via its direct antagonism of vascular α_2-adrenoceptors. No bradycardia was observed with MED+V, likely due to lack of baroreceptor responses in the context of normal systemic vascular resistance [5]. The observed fall in heart rate with MED+V can likely be attributed to reduced sympathetic tone. These findings are consistent with previous studies on horses, in which vatinoxan was administered simultaneously with single bolus doses of medetomidine [9], detomidine [10] or romifidine [11]. Noticeably, the baseline MAP readings in this study were unexpectedly high, which may reflect a stress response of these unsedated horses, standing in stocks prior to the start of the experiment. Similarly, stress and consequent moving of horses could at least partially explain the relatively large variation in the CI measurements at 120 min. The arterial catheters functioned normally and LiDCO system displayed no errors during CI measurement. Certain drugs, including α_2-agonists, are known to interact with lithium sensors in vitro [21]. The concentrations of dexmedetomidine in this study, however, were markedly lower than those that caused interference with the sensor in vitro [21], thus dexmedetomidine as a source of bias in cardiac output measurement is therefore unlikely. Nevertheless, comparisons of the post-drug blood pressure values to baseline and interpretation of CI measurements at 120 min should be treated with caution.

Decreased Qt is a recognised effect of medetomidine in horses [4,6,22]. In dogs, dexmedetomidine did not have a direct myocardial depressant effect; the decrease in cardiac output and stroke volume were likely consequences of its other haemodynamic effects [23]. Thus, it is plausible that as SVR remained stable and bradycardia did not ensue with MED+V, CI also remained unchanged. The increased CVP initially seen after medetomidine and its attenuation by vatinoxan are in line with the other haemodynamic findings of this study. Increased CVP suggests increased preload, which has been attributed to peripheral and pulmonary vasoconstriction caused by α_2-agonists [6]. Interestingly, some evidence also suggests that equine saphenous veins may express adrenergic receptors [24], in which case medetomidine and vatinoxan may additionally have direct effects on venous walls. Our CVP observations agree with a previous study that combined vatinoxan with an i.v. bolus dose of detomidine in horses [10]. In contrast, increased CVP has not been consistently observed after medetomidine [4]. This could be partially explained by the use of a smaller dose of medetomidine than in our study, as the cardiovascular effects of medetomidine seem to be dose-dependent within a certain range [6]. Finally, the cardiovascular suppression in horses during CRI of medetomidine has been reported to be minor after the changes following the medetomidine loading dose have waned [2]. Although there was also a tendency in this study for most of these haemodynamic changes to occur early after the medetomidine loading dose, these changes could be alleviated by vatinoxan.

In this study, vatinoxan had no detectable effect on the observed transient (although minor) decrease in PaO_2 after medetomidine. Nevertheless, small but significant decreases in PvO_2 after medetomidine were observed, and were found to be alleviated by vatinoxan, similarly to previous reports [4,22]. Furthermore, vatinoxan improved the O_2ER, which describes the amount of oxygen removed from blood by tissues. The

oxygen extraction ratio increases in situations such as tissue hypoxia and increased metabolic oxygen demand [25]. Thus, improved O_2ER probably reflects better tissue perfusion in horses receiving vatinoxan. The improved PvO_2 and O_2ER with vatinoxan paralleled the improved haemodynamic parameters (*i.e.* SVRI and CI) during the CRI, as presumed. The improved oxygenation and tissue perfusion may become clinically important in sedation protocols for surgical procedures or in horses that have a systemic illness and compromised cardiovascular function, such as horses with severe colic.

α_2-agonists are known to increase plasma glucose concentrations by decreasing insulin secretion *via* activation of α_2-adrenoceptors located on pancreatic β-cells, and this was observed in this study as well with medetomidine, as also previously reported [26]. Also, this effect was alleviated by vatinoxan, probably *via* blockade of pancreatic α_2-adrenoceptors, as seen in dogs [27].

In this study, medetomidine decreased borborygmi (as assessed by auscultation) as has been reported previously [26]. α_2-adrenoceptors with an inhibitory effect on motility are expressed in equine jejunum [28]. In addition, the equine caecum and colon are postulated to be more sensitive to the effects of medetomidine than the jejunum [29], which is supported by a previous observation of decreased faecal output after medetomidine for 3.5–5 h [26]. This increases the risk for post-sedation colon impaction and colic. In our study, vatinoxan alleviated the decrease in gastrointestinal borborygmi scores throughout the observation period, and the scores were restored to baseline levels sooner in the presence of vatinoxan. This is consistent with previous studies where vatinoxan prevented [10] and reversed [12] gastrointestinal hypomotility after administration of detomidine. Direct antagonism of vatinoxan at the level of intestinal α_2-adrenoceptors could explain the improved gastrointestinal motility. Thus, vatinoxan may reduce the risk of post-sedation colic in horses, especially after more prolonged sedation with a CRI of medetomidine.

All horses were sedated after administration of medetomidine. Vatinoxan slightly yet significantly decreased the mean sedation scores during the first 20 min. The decreased sedation scores may at least in part be explained by the differences in the plasma concentrations of dexmedetomidine between the two treatments. Vatinoxan has previously been reported to enhance the distribution and clearance of i.v. bolus doses of detomidine [10] and romifidine [11] in horses, most likely through changes in haemodynamics. In the aforementioned studies, vatinoxan decreased the mean sedation scores [11] or areas under the time-sedation score curves, albeit the maximum sedation scores were unaffected [10]. Furthermore, in dogs the distribution of an i.v. bolus dose of dexmedetomidine was increased by concomitant vatinoxan mainly during the first 10 min, after which the elimination curves of dexmedetomidine with and without vatinoxan appeared parallel [30]. Although pharmacokinetics were not investigated in such detail in this study, the sedation scores differed between the treatments only in the first 20 min following the administration of vatinoxan and the loading dose of medetomidine. This supports the concept of vatinoxan promoting the early distribution of dexmedetomidine. In horses, the half-lives of medetomidine and dexmedetomidine are short (11–29 min [2,26] and 19–29 min [3,22] respectively). Whilst, the pharmacokinetic steady state of medetomidine is attained within approximately 30 min using a CRI and a smaller loading dose (5 μg/kg) than that used in this study [2]. In comparison, the half-life of vatinoxan in horses has been reported to be markedly longer than that of (dex)medetomidine; over 2 h when given alone [11]. In this study, vatinoxan half-life was approximately 75 min (mean) in the presence of medetomidine. Thus, it is plausible that using a medetomidine CRI, steady state was achieved in our horses, and, furthermore, that the antagonistic effect of the single dose of vatinoxan persisted during this phase without major impact on the sedation level.

A limitation of this study was the use of healthy, unstimulated animals. The effects of vatinoxan on sedation quality and on the potential analgesic effect of medetomidine when a nociceptive stimulus is applied, remains to be investigated.

In conclusion, vatinoxan administered simultaneously with a loading dose of medetomidine improved cardiovascular and gastrointestinal function during a CRI of medetomidine in healthy horses. This was achieved without noticeable side effects or clinically relevant effects on sedation scores. By improving the haemodynamics of horses during sedation with medetomidine, vatinoxan could also improve tissue perfusion during sedation. Improved gastrointestinal motility may reduce the risk of post-sedation colic. The safety of vatinoxan in clinically ill horses and its effects on the analgesic efficacy of α_2-agonists warrant further studies.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

This study was approved by the National Animal Experiment Board of Finland (No. ESAVI/4789/04.10.07/2014).

Owner informed consent

Not applicable.

Sources of funding

Financial support was provided by Vetcare Ltd. (Mäntsälä, Finland) for the study expenses; materials and drugs.

Acknowledgements

The authors thank Prof Paula Larenza for her contribution to the study design.

Authorship

H. Tapio and M. Raekallio contributed to study design, study execution, data analysis and interpretation and preparation of the manuscript. A. Mykkänen contributed to study design, study execution and preparation of the manuscript. S. Männikkö and M. Scheinin contributed to data analysis and interpretation, and preparation of the manuscript. R. Bennett contributed to study execution and preparation of the manuscript. O. Vainio contributed to study design and preparation of the manuscript. All authors gave their final approval of the manuscript.

Manufacturers' addresses

[a]AstraZeneca, Wedel, Germany.
[b]B. Braun, Melsungen, Germany.
[c]Datex Ohmeda, Madison, Wisconsin, USA.
[d]Laboratoires Pharmaceutiques Vygon, Ecouen, France.
[e]Becton-Dickinson Critical Care Systems, Singapore.
[f]LiDCO, London, UK.
[g]Radiometer Medical, Brønshøj, Denmark.
[h]Vetcare, Helsinki, Finland.
[i]Merck & Co, Kenilworth, New Jersey, USA.
[j]Certara, Princeton, New Jersey, USA.
[k]Waters Co, Milford, Massachusetts, USA.
[l]Toronto Research Chemicals, Toronto, Ontario, Canada.
[m]R&D Systems, Minneapolis, Minnesota, USA.
[n]Chiral Technologies Europe, Illkirch, France.
[o]MDS Sciex, Concord, Ontario, Canada.
[p]SAS Institute Inc., Cary, North Carolina, USA.
[q]IBM Finland, Espoo, Finland.

References

1. Pertovaara, A. (1993) Antinociception induced by α_2-adrenoceptor agonists, with special emphasis on medetomidine studies. *Prog. Neurobiol.* **40**, 691-709.

2. Bettschart-Wolfensberger, R., Clarke, K.W., Vainio, O., Aliabadi, F. and Demuth, D. (1999) Pharmacokinetics of medetomidine in ponies and elaboration of a medetomidine infusion regime which provides a constant level of sedation. *Res. Vet. Sci.* **67**, 41-46.
3. Ranheim, B., Risberg, A.I., Spadavecchia, C., Landsem, R. and Haga, H.A. (2015) The pharmacokinetics of dexmedetomidine administered as a constant rate infusion in horses. *J. Vet. Pharmacol. Ther.* **38**, 93-96.
4. Bettschart-Wolfensberger, R., Bettschart, R.W., Vainio, O., Marlin, D. and Clarke, K.W. (1999) Cardiopulmonary effects of a two hour medetomidine infusion and its antagonism by atipamezole in horses and ponies. *Vet Anaesth. Analg.* **26**, 8-12.
5. Flacke, J.W., Flacke, W.E., Bloor, B.C. and McIntee, D.F. (1990) Hemodynamic effects of dexmedetomidine, an α_2-adrenergic agonist, in autonomically denervated dogs. *J. Cardiovasc. Pharmacol.* **16**, 616-623.
6. Yamashita, K., Tsubakishita, S., Futaok, S., Ueda, I., Hamaguchi, H., Seno, T., Katoh, S., Izumisawa, Y., Kotani, T. and Muir, W.W. (2000) Cardiovascular effects of medetomidine, detomidine and xylazine in horses. *J. Vet. Med. Sci.* **62**, 1025-1032.
7. Rezende, M.L., Grimsrud, K.N., Stanley, S.D., Steffey, E.P. and Mama, K.R. (2015) Pharmacokinetics and pharmacodynamics of intravenous dexmedetomidine in the horse. *J. Vet. Pharmacol. Ther.* **38**, 15-23.
8. Clineschmidt, B.V., Pettibone, D.J., Lotti, V.J., Hucker, H.B., Sweeney, B.M., Reiss, D.R., Lis, E.V., Huff, J.R. and Vacca, J. (1988) A peripherally acting α_2-adrenoceptor antagonist: L-659,066. *J. Pharmacol. Exp. Ther.* **245**, 32-40.
9. Bryant, C.E., Thompson, J. and Clarke, K.W. (1998) Characterisation of the cardiovascular pharmacology of medetomidine in the horse and sheep. *Res. Vet. Sci.* **65**, 149-154.
10. Vainionpää, M.H., Raekallio, M.R., Pakkanen, S.A., Ranta-Panula, V., Rinne, V.M., Scheinin, M. and Vainio, O.M. (2013) Plasma drug concentrations and clinical effects of a peripheral α_2-adrenoceptor antagonist, MK-467, in horses sedated with detomidine. *Vet Anaesth. Analg.* **40**, 257-264.
11. de Vries, A., Pakkanen, S.A., Raekallio, M.R., Ekiri, A., Scheinin, M., Taylor, P.M. and Vainio, O.M. (2016) Clinical effects and pharmacokinetic variables of romifidine and the peripheral α_2-adrenoceptor antagonist MK-467 in horses. *Vet Anaesth. Analg.* **43**, 599-610.
12. Tapio, H.A., Raekallio, M.R., Mykkänen, A., Mama, K., Mendez-Angulo, J.L., Hautajärvi, H. and Vainio, O.M. (2018) Effects of MK-467 hydrochloride and hyoscine butylbromide on cardiorespiratory and gastrointestinal changes induced by detomidine hydrochloride in horses. *Am. J. Vet. Res.* **79**, 376-387.
13. Tapio, H., Arguelles, D., Gracia-Calvo, L.A. and Raekallio, M. (2017) Modified technique for common carotid artery transposition in standing horses. *Vet. Surg.* **46**, 52-58.
14. Hallowell, G.D. and Corley, K.T. (2005) Use of lithium dilution and pulse contour analysis cardiac output determination in anaesthetized horses: a clinical evaluation. *Vet Anaesth. Analg.* **32**, 201-211.
15. Rohrbach, H., Korpivaara, T., Schatzmann, U. and Spadavecchia, C. (2009) Comparison of the effects of the α_2-agonists detomidine, romifidine and xylazine on nociceptive withdrawal reflex and temporal summation in horses. *Vet Anaesth. Analg.* **36**, 384-395.
16. Mama, K.R., Grimsrud, K., Snell, T. and Stanley, S. (2009) Plasma concentrations, behavioural and physiological effects following intravenous and intramuscular detomidine in horses. *Equine Vet. J.* **41**, 772-777.
17. Haskins, S., Pascoe, P.J., Ilkiw, J.E., Fudge, J., Hopper, K. and Aldrich, J. (2005) Reference cardiopulmonary values in normal dogs. *Comp. Med.* **55**, 156-161.
18. Clerbaux, T., Gustin, P., Detry, B., Cao, M.L. and Frans, A. (1993) Comparative study of the oxyhaemoglobin dissociation curve of four mammals: man, dog, horse and cattle. *Comp. Biochem. Physiol. Comp. Physiol.* **106**, 687-694.
19. Boyd, C.J., McDonell, W.N. and Valliant, A. (1991) Comparative hemodynamic effects of halothane and halothane-acepromazine at equipotent doses in dogs. *Can. J. Vet. Res.* **55**, 107-112.
20. Olsen, E., Skovgaard Pedersen, T.L., Robinson, R. and Haubro Andersen, P. (2016) Accuracy and precision of oscillometric blood pressure in standing conscious horses. *J. Vet. Emerg. Crit. Care.* **26**, 85-92.
21. Ambrisko, T.D., Kabens, R. and Moens, Y. (2013) Influence of drugs on the response characteristics of the LiDCO sensor: an in vitro study. *Br. J. Anaesth.* **110**, 305-310.
22. Bettschart-Wolfensberger, R., Freeman, S.L., Bowen, I.M., Aliabadi, F.S., Weller, R., Huhtinen, M. and Clarke, K.W. (2005) Cardiopulmonary effects and pharmacokinetics of i.v. dexmedetomidine in ponies. *Equine Vet. J.* **37**, 60-64.
23. Flacke, W.E., Flacke, J.W., Blow, K.D., McIntee, D.F. and Bloor, B.C. (1992) Effect of dexmedetomidine, an α_2-adrenergic agonist, in the isolated heart. *J. Cardiothorac. Vasc. Anesth.* **6**, 418-423.
24. Bryant, C.E. and Clarke, K.W. (1996) The actions of medetomidine may not be mediated exclusively by α_2-adrenoceptors in the equine saphenous vein. *J. Vet. Pharmacol. Ther.* **19**, 124-129.
25. Park, J., Kim, S., Lee, S., Lee, E., Han, K., Moon, S. and Hong, Y. (2015) Initial low oxygen extraction ratio is related to severe organ dysfunction and high in-hospital mortality in severe sepsis and septic shock patients. *J. Emerg. Med.* **49**, 261-267.
26. Grimsrud, K.N., Mama, K.R., Steffey, E.P. and Stanley, S.D. (2012) Pharmacokinetics and pharmacodynamics of intravenous medetomidine in the horse. *Vet Anaesth. Analg.* **39**, 38-48.
27. Restitutti, F., Raekallio, M., Vainionpaa, M., Kuusela, E. and Vainio, O. (2012) Plasma glucose, insulin, free fatty acids, lactate and cortisol concentrations in dexmedetomidine-sedated dogs with or without MK-467: a peripheral α_2-adrenoceptor antagonist. *Vet. J.* **193**, 481-485.
28. Zullian, C., Menozzi, A., Pozzoli, C., Poli, E. and Bertini, S. (2011) Effects of α_2-adrenergic drugs on small intestinal motility in the horse: an in vitro study. *Vet. J.* **187**, 342-346.
29. Sasaki, N., Yoshihara, T. and Hara, S. (2000) Difference in the motile reactivity of jejunum, cecum, and right ventral colon to xylazine and medetomidine in conscious horses. *J. Equine Sci.* **11**, 63-68.
30. Honkavaara, J., Restitutti, F., Raekallio, M., Salla, K., Kuusela, E., Ranta-Panula, V., Rinne, V., Vainio, O. and Scheinin, M. (2012) Influence of MK-467, a peripherally acting α_2-adrenoceptor antagonist on the disposition of intravenous dexmedetomidine in dogs. *Drug Metab. Dispos.* **40**, 445-449.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Summary in Portuguese.
Supplementary Item 1: Sedation scores.
Supplementary Item 2: Borborygmi scores.
Supplementary Item 3: Calculated indices.