The Veterinary Journal 202 (2014) 489–497

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Antinociceptive effects of three escalating dexmedetomidine and lignocaine constant rate infusions in conscious horses

Å. Risberg [a,*], C. Spadavecchia [b], B. Ranheim [c], R. Krontveit [d], H.A. Haga [a]

[a] Department of Veterinary Medicine and Life Sciences, Norwegian University of Life Sciences, 0454 Oslo, Norway
[b] Vetsuisse Faculty, Department of Clinical Veterinary Sciences, 3012 Berne, Switzerland
[c] Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine and Life Sciences, Norwegian University of Life Sciences, 0454 Oslo, Norway
[d] Faculty of Veterinary Medicine and Life Sciences, Centre for Epidemiology and Biostatistics, Norwegian University of Life Sciences, 0454 Oslo, Norway

ARTICLE INFO

Article history:
Accepted 5 September 2014

Keywords:
Equine
Antinociception
Dexmedetomidine
Lignocaine
Constant rate infusion

ABSTRACT

Dexmedetomidine and lignocaine IV are used clinically to provide analgesia in horses. The aims of this study were to investigate the antinociceptive effects, plasma concentrations and sedative effects of 2, 4 and 6 μg/kg/h dexmedetomidine IV, with a bolus of 0.96 μg/kg preceding each continuous rate infusion (CRI), and 20, 40 and 60 μg/kg/min lignocaine IV, with a bolus of 550 μg/kg preceding each CRI, in 10 Swiss Warmblood horses. Electrically elicited nociceptive withdrawal reflexes were evaluated by deltoid muscle electromyography. Nociceptive threshold and tolerance were determined by electromyography and behaviour following single and repeated stimulation. Plasma concentrations of drugs were determined by liquid chromatography and mass spectrometry. Sedation was scored on a visual analogue scale. Dexmedetomidine increased nociceptive threshold to single and repeated stimulation for all CRIs, except at 2 μg/kg/h, where no increase in single stimulation nociceptive threshold was observed. Dexmedetomidine increased nociceptive tolerance to single and repeated stimulation at all CRIs. There was large individual variability in dexmedetomidine plasma concentrations and levels of sedation; the median plasma concentration providing antinociceptive effects to all recorded parameters was 0.15 ng/mL, with a range from <0.02 ng/mL (below the lower limit of quantification) to 0.25 ng/mL. Lignocaine increased nociceptive threshold and tolerance to single and repeated stimulation at CRIs of 40 and 60 μg/kg/min, corresponding to plasma lignocaine concentrations >600 ng/mL. Only nociceptive tolerance to repeated stimulation increased at 20 μg/kg/min lignocaine. Lignocaine at 40 μg/kg/min and dexmedetomidine at 4 μg/kg/h were the lowest CRIs resulting in consistent antinociception. Lignocaine did not induce significant sedation.

Introduction

Dexmedetomidine, the active enantiomer of the racemic mixture medetomidine, is currently the most specific α-2 agonist in clinical use. α-2 Agonists are used as constant rate infusions (CRI) during anaesthesia to provide antinociception and to reduce the requirement for inhaled anaesthetic agents in horses (Bettschart-Wolfensberger et al., 2001, 2011; Gozalo-Marcilla et al., 2013a, 2013b, 2013c). Antinociception with dexmedetomidine is mediated primarily through α-2a receptors in the spinal cord (Yaksh, 1985; Takano and Yaksh, 1991; Guo et al., 1996; Zhao et al., 2013), but an effect within the locus coeruleus also produces antinociception (Guo et al., 1996; Funai et al., 2013). The antinociceptive effect of dexmedetomidine has been reported in dogs (Valtolina et al., 2009; van Oostrom et al., 2011) and cats (Slingsby et al., 2009, 2010; Porters et al., 2014) but not in conscious horses.

Lignocaine is used to provide intra-operative and post-operative analgesia, to reduce the requirements for inhaled anaesthetic agents (Doherty and Frazier, 1998; Dzikiti et al., 2003; Murrell et al., 2005; Ringer et al., 2007) and to prevent ileus after colic surgery in horses (Torfs et al., 2009). Although few studies have evaluated the antinociceptive effects of systemic lignocaine in conscious horses, Robertson et al. (2005) reported an increased thermal threshold and de Souza et al. (2012) reported a delayed response to electrical stimulation when lignocaine was combined with xylazine.

The pharmacokinetic profiles following lignocaine infusions in conscious and anaesthetised horses have been determined (Feary et al., 2005, 2006), and the plasma concentrations for reducing the minimal alveolar concentration of inhalational anaesthetics, as well as plasma concentrations producing toxic side effects in conscious horses, are known (Meyer et al., 2001; Waxman et al., 2012). However, the minimum infusion rate and corresponding plasma

* Corresponding author. Tel.: +47 22964500.
E-mail address: aase.risberg@nmbu.no (Å. Risberg).

http://dx.doi.org/10.1016/j.tvjl.2014.09.007
1090-0233/

concentrations of lignocaine necessary to provide antinociception in conscious horses have not been described.

The nociceptive withdrawal reflex (NWR) is a spinal reflex elicited when tissue damage or risk of tissue damage occur. The NWR may be quantified precisely when evoked by electrical stimulation. Electromyography (EMG) records the electrical activity in contracting muscles and the root mean square (RMS) amplitude of the EMG may be used to quantify the size of the EMG response (Reaz et al., 2006). The NWR threshold is the stimulus intensity needed to elicit an NWR, while the nociceptive tolerance is the maximum stimulus intensity tolerated by an individual. The NWR is used as a neurophysiological tool to investigate nociceptive processing and pharmacological modulation of nociception in human beings and veterinary species (Willer, 1977; Willer and Bathien, 1977; Arendt-Nielsen et al., 1995; Spadavecchia et al., 2005; Bergadano et al., 2009). An increase in NWR threshold and nociceptive tolerance, and a decreasing reflex response following administration of a drug, may be used as quantitative indicators of the drug's antinociceptive effect (Ercoli and Lewis, 1945; Spadavecchia et al., 2005).

The main aim of this study was to evaluate the antinociceptive effects of three escalating dexmedetomidine and lignocaine infusion rates in conscious horses using the NWR model. We also aimed to determine the minimum infusion rate and plasma concentration necessary to provide antinociception, as well as the sedative effect of dexmedetomidine and lignocaine during all infusion rates.

Materials and methods

Study design and study population

Eleven Swiss Warmblood geldings with mean ± standard deviation (SD) weight and age of 602 ± 39 kg and 15.4 ± 4.5 years were included in the study. Nine horses were used in a randomised, balanced, crossover design, with a wash out period of minimum 14 days, while one horse was used for the lignocaine experiment only and one horse was used for the dexmedetomidine experiment only.

Horses were familiarised with the experimental room on the afternoon before the experiment, and placement sites for electrodes and jugular catheters were clipped of hair. On the morning of the experiments, horses were exercised for 30–45 min. All horses underwent a physical examination on the day of the experiment and were judged to be clinically healthy. Horses were placed in stocks during the entire experimental session (3–4 h). Experiments were approved by the Committee of Animal Experimentation, Berne, Switzerland (BE 67/10; date of approval 24 June 2010).

Electrical stimulation and recording

Electrical stimulation and recording were performed with a purpose-built computerised system (Spadavecchia et al., 2002). Single stimulation consisted of a train of five 1 ms constant current square wave pulses delivered at a frequency of 200 Hz. Electromyographic activity was recorded from 100 ms prior to until 400 ms after the stimulus; the total recording time was 500 ms (sampling frequency 1 kHz) for a single stimulation. An electromyogram (EMG) burst with a minimum root mean square (RMS) amplitude of 30 μV within the post-stimulation epoch of 40–200 ms was considered to be the NWR threshold.

Repeated stimulations consisted of single stimulations applied 10 times at 5 Hz over 2 s. Electromyographic activity was recorded from 500 ms prior to stimulation until 1500 ms thereafter; total recording time was 4000 ms (sampling frequency 1 kHz).

Electrode placement

The area over the right lateral palmar digital nerve was clipped of hair, cleaned and two self-adhesive electrodes (Neuroline 700, Ambu) for electrical stimulation were placed on the skin. The distance between the electrodes was a minimum of 20 mm. A ground electrode was placed on the horse's back. Surface electromyograms of the deltoid muscle of the right forelimb were bipolarly recorded using pairs of self-adhesive electrodes (Synapse, Ambu) placed parallel to the muscle fibres at a minimum of 20 mm apart. The resistance between the stimulation electrodes was measured using a multimeter (113 True RMS multimeter, Fluke) prior to each experimental session and maintained at <3 kΩ. Flexible leads were connected to the electrodes; electrodes and leads were secured with adhesive bandages to avoid displacement.

Table 1
Scoring system for behavioural reactions during single stimulation (modified from Rohrbach et al., 2009).

Unsedated horse		Sedated horse	
0	No reaction	0	No reaction
1	Slight brisk whole body reaction	1	Slight localised reaction
2	Moderate brisk whole body reaction	2	Moderate localised reaction and movement of the head
3	Prompt whole body reaction and delayed lifting of the stimulated limb	3	Prompt whole body reaction and delayed lifting of the stimulated limb
4	Immediate lifting of the limb	4	Immediate lifting of the limb
5	Vigorous whole body reaction	5	Vigorous whole body reaction

Behaviour

Behavioural responses (reactions) following single and repeated stimulations were scored according to Tables 1 and 2, respectively, using systems modified from Rohrbach et al. (2009).

Electrical stimulation and data acquisition

After electrode placement, spontaneous electromyographic activity was recorded to ensure correct acquisition of the system. Single stimuli in steps of 1 mA were given until a reaction score of 1 was achieved, in order to acquaint the horse to the sensation of the stimulus. The stimulator was activated manually when the horse was standing quietly without head or limb movement. Recordings were repeated if they coincided with any movement of the horse unrelated to the stimulations. Stimulation series consisted of single and repeated stimulations given before drug administration (baseline) and 15 min after starting each of the three CRIs. An additional stimulation series 30 min following discontinuation of the last CRI was completed in six horses.

Drug and crystalloid administration

Catheters (14 G, Secalon-T, Argon) were placed in the right jugular vein for administration of the treatment drug and crystalloids, and in the left jugular vein for blood collection. Dexmedetomidine (Dexdomitor, Orion Pharma) diluted in 0.9% NaCl to 0.05 mg/mL or lignocaine (Lignocaine 3%, Christoffel-Apotheke, A. Grogg) were administered by syringe driver (Ivac Diprifusor, Alaris). Drug administration was started with a bolus dose and the CRIs were given in escalating order interspersed by the same bolus dose to rapidly achieve a new plasma level. For dexmedetomidine, boluses of 0.96 μg/kg were administered by hand over 1 min and the infusion rates were 2, 4 and 6 μg/kg/h. The sizes of boluses were calculated on the basis of available pharmacokinetic data (Bettschart-Wolfensberger et al., 2005). Boluses of 550 μg/kg lignocaine were administered over 10 min and infusion rates were 20, 40 and 60 μg/kg/min. Boluses were calculated based on available pharmacokinetic data for conscious horses (Feary et al., 2005). Crystalloids were administered at a rate of 300–500 mL/horse/h.

Blood sampling

A volume of 10 mL blood was discarded before 10 mL was collected according to Table 2 for lignocaine analysis and according to Table 3 for dexmedetomidine analysis. Blood was transferred to heparinised tubes and centrifuged, then the plasma was immediately frozen in plastic cryotubes at –20 °C and shipped on dry ice. Dexmedetomidine in plasma samples was analysed at the University of Turku, CRST Bioanalytics, Turku, Finland, using liquid chromatography with mass spectral detection. The analytical method was validated in-house and found to be linear from 0.02 to 10.0 ng/mL; the lower limit of quantification (LLOQ) was 0.02 ng/mL.

Plasma lignocaine samples were analysed at the Norwegian University of Life Sciences using liquid chromatography with mass spectral detection (Bo et al., 1999; Maes et al., 2007). The limit of detection was 2 ng/mL and the lower limit of quantification was 5 ng/mL. The results from one horse were excluded from the analysis due to inexplicable high lignocaine concentrations in the samples.

Nociception

To determine the nociceptive threshold, single stimulations were applied from 1 mA in increments of 1 mA until a minimum reaction score of 2 corresponding to a NWR was observed. Stimulation at this intensity was repeated twice. A NWR had