



Dexmedetomidine for balanced anaesthesia in horses

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“Choose a job you love, and you will never have to work a day in your life”.

Confucius (551-479 B.C.)

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List of abbreviations

ABP	Arterial blood pressure
ANOVA	Analysis of variance
ASA	American Society of Anesthesiologists
CaO ₂	Arterial oxygen content
CC	Constant current
CEPEF	Confidential Enquiry into Perioperative Equine Fatalities
CI	Cardiac indexed to weight
CNS	Central nervous system
CRI	Constant rate infusion
CvO ₂	Venous oxygen content
CYP450	Cytochrome 450
DAP	Diastolic arterial pressure
DO ₂	Oxygen delivery
DO ₂ I	Oxygen delivery indexed to weight
ECG	Electrocardiogram
F _E '	Expired fraction
FiO ₂	Inspired oxygen fraction
GABA	Gamma-aminobutyric acid
HALO	Halothane
Hb	Haemoglobin
HR	Heart rate
ICU	Intensive care unit
IM	Intramuscular
IPPV	Intermittent positive pressure ventilation
IV	Intravenous
ISO	Isoflurane
LC	Locus coeruleus
LiDCO	Lithium dilution cardiac output
MAC	Minimum alveolar concentration
MAC _{NM}	Minimum alveolar concentration no movement
MAP	Mean arterial pressure

NMDA	N-methyl-D-aspartate
PaCO ₂	Arterial partial pressure of carbon dioxide
PaO ₂	Arterial partial pressure of oxygen
P _B	Barometric pressure
PCV	Packed cell volume
PEEP	Positive end-expiratory pressure
PIVA	Partial intravenous anaesthesia
POI	Post-operative ileus
Q̇ _t	Cardiac output
RAP	Right atrial pressure
RR	Respiratory rate
SAP	Systolic arterial pressure
SD	Standard deviation
SEVO	Sevoflurane
SV	Stroke volume
SVI	Stroke volume indexed to weight
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance indexed to weight
TCI	Target-controlled infusions
TIVA	Total intravenous anaesthesia
t _{1/2}	Terminal half-life
Ṡ/Ḡ	Ventilation/perfusion
V _{ss}	Steady state volume of distribution

General introduction

General anaesthesia in horses carries a higher risk of mortality compared to other species. The increased incidence of intraoperative complications and death is partly related to the dose dependent cardiovascular depression induced by inhalation anaesthetics, commonly used for long procedures. In order to reduce the required amount of inhalants and associated side effects, combinations of different anaesthetic drugs are often incorporated in the so-called 'balanced anaesthetic protocols'. Nowadays, this term has reached new dimensions with the use of less soluble inhalant anaesthetics in combination with short-acting intravenous (IV) anaesthetic drugs. In equine anaesthesia, the aims of balanced anaesthesia are to preserve an optimal intraoperative cardiopulmonary function and to assure good recoveries without adverse events.

Perioperative fatalities associated with equine general anaesthesia

The overall incidence of anaesthetic and sedation-related death has been reported to be 0.17 in dogs and 0.24% in cats, increasing to 1.33 and 1.40% respectively in sick patients (Brodbelt et al. 2007, 2008). These values are substantially higher than those seen in human anaesthesia: a review study reported an overall mortality rate of 0.008% that may increase up to 0.05% in ASA IV (American Society of Anesthesiologists) patients (Lagasse 2002). Different studies have been carried out in an attempt to determine the mortality rate associated with general anaesthesia in equines, although it remains difficult to determine whether death is either related purely to anaesthesia, or rather to the result of underlying diseases and/or surgical complications. Tevik (1983) reported a perioperative mortality of 0.8% in horses undergoing different types of surgery. The surgical/anaesthetic death rate was 0.63% in horses undergoing elective procedures, although only 0.08% was directly attributable to anaesthesia (Mee et al. 1998a). Young & Taylor (1993) described a 0.68% incidence of anaesthetic-related deaths in horses undergoing orthopaedic surgery, radiography or minor soft tissue surgery. Higher mortality rates (31.4%) were noticed for emergency procedures (Mee et al. 1998b). More recently, a study performed in a private referral practice reported the prevalence of equine fatalities directly related to anaesthesia to be 0.12%, increasing to 0.24% when horses euthanized or dying within seven days after general anaesthesia were included (Bidwell et al. 2007). However, several factors contributed to this lower fatality rate. Most of the horses were healthy, the duration of anaesthesia was usually shorter than sixty minutes and the anaesthetists were familiar with the standard protocol used in their practice.

The CEPEF (Confidential Enquiry into Perioperative Equine Fatalities) is the largest observational study of equine fatalities. This study was performed over a period of six years

and included data collected from 41,824 horses undergoing general anaesthesia in 129 different equine clinics (Johnston et al. 2002). During anaesthesia or within a period of seven days after anaesthesia, an overall death rate of 1.9% was found. This overall number was reduced to 0.9% when only non-colic horses were included, but increased to 11.7% in colic horses. This report was also useful to identify major important risk factors other than emergency abdominal surgery, such as age, type and time of surgery and drugs used for premedication, induction and maintenance of anaesthesia. Induction of anaesthesia with volatile agents, night-time and weekend surgical procedures and fracture repairs were associated with higher risks as well. Furthermore, the maintenance of anaesthesia with volatile agents carried a much higher risk of death (0.99%) compared to total intravenous anaesthetic (TIVA) protocols (0.31%). Intravenous agents were suggested to produce less cardiorespiratory depression compared to volatile agents, resulting in a better outcome (Taylor et al. 1998). These data suggest that volatile agent anaesthesia can significantly contribute to the high anaesthetic mortality in equines, mostly due to the occurring cardiovascular effects (Steffey & Howland 1978). However, TIVA is routinely only applied in horses anaesthetized for less than forty five minutes (Hubbell 2007), mainly because of the risk of drug accumulation and associated side effects. Although the numbers in the CEPEF study were insufficient for a proper statistical evaluation, two out of sixty two horses (3.2%) anaesthetized with a TIVA protocol for more than ninety minutes died (Johnston et al. 2002).

Johnston et al. (2002) also demonstrated that 33% of the deaths were due to cardiac arrest (including cardiovascular collapse). Furthermore, 32% of the horses were euthanized due to occurring fractures or myopathies during the recovery period with the remaining 35% of deaths related to a wide range of etiologies. Cardiac arrest is often the end-result of pre-existing, pronounced cardiovascular depression. Moreover, the association between myopathy and hypotension has been well established in horses. Myopathy develops as a result of inadequate muscle perfusion during anaesthesia, caused by decreases in blood pressure and cardiac output (\dot{Q}_t), combined with increased intracompartmental pressures in dependent limb muscle (Grandy et al. 1987; Lindsay et al. 1989). It also seems likely that some of the fractures reported in the CEPEF study were related to muscle dysfunction and/or myopathy caused by an inadequate oxygen delivery during anaesthesia. Indeed, Edner et al. (2005) demonstrated with the microdialysis technique the occurrence of an anaerobic metabolic response, even in muscles of healthy horses undergoing long-term inhalation anaesthesia. Horses with myopathy are supposed to have difficulties or are even unable to stand, leading to excitation and prolonged recumbency, causing further muscle damage. Moreover, fractures

may occur during unsuccessful attempts to stand due to muscle weakness (Lindsay et al. 1989). Although myopathies have different intensities and are not always fatal, the prognosis remains very poor when a generalized myopathy is present in equine patients. Myopathy was also the main reported cause of death in the other study including 1314 healthy horses (Young & Taylor 1993).

The studies of Young & Taylor (1993) and Johnston et al. (2002) included a large number of horses and showed that perioperative death in horses was closely linked with inadequate tissue oxygen delivery. This confirms the importance of maintaining a good cardiovascular function during anaesthesia, not only in high risk patients such as the colic horse, but also in healthy horses. Consequently, an adequate monitoring of anaesthetized horses must allow the anaesthetist to detect cardiovascular depression on time.

Cardiovascular depression due to inhalational agents

At the present moment, volatile anaesthetics are widely used in horses because they are the most controllable method for producing general anaesthesia (Steffey 2009). However, these agents are not free of side effects since they induce a 'dose dependent' cardiovascular depression (Clarke 2008). Halothane has been widely used since its introduction into clinical practice in the late 1950s by Dr. Leslie Hall (Clarke 2008) and enabled many of the advances in veterinary surgery. This drug is no longer available in most countries. Halothane produces bradycardia by increasing vagal tone, depressing sino-atrial and atrioventricular activity. Moreover, it reduces myocardial contractility and \dot{Q}_t and sensitizes the heart to catecholamines, which may lead to arrhythmias. Arterial blood pressure (ABP) also decreases but there is some vasoconstriction and at one minimum alveolar concentration (MAC), hypotension is less pronounced than anticipated (Clarke 2008). Isoflurane, a greater respiratory depressant than halothane (Steffey & Howland 1980), is nowadays the most commonly used licensed inhalation anaesthetic agent in horses. This volatile agent reduces systemic vascular resistance (SVR), inducing a marked vasodilation and a decreased ABP. On the other hand, isoflurane maintains heart rate (HR) better and has less effect on myocardial contractility compared to halothane, preserving a better \dot{Q}_t and peripheral perfusion. Sevoflurane, a newer volatile agent, has also been commercialized for dogs and is on the 'list of essential substances' for food producing horses with a six-month withdrawal (Clarke 2008). Its use has been reported in different experimental (Aida et al. 1994; Rezende et al. 2011) and clinical studies (Matthews et al. 1999) and cardiopulmonary depressant side effects have been observed. Sevoflurane anaesthetized horses will breathe at lower frequencies and

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can become hypercapnic (Steffey et al. 2005a), which is similar to findings in horses anaesthetized with isoflurane (Steffey & Howland 1980). Sevoflurane also induces a fall in the SVR and ABP, without changes in cardiac contractility or HR. Although desflurane is not licensed for use in animals, this volatile agent has been studied in experimental horses (Clarke et al. 1996; Tendillo et al. 1997; Bettschart-Wolfensberger et al. 2001; Steffey et al. 2005b). It produces profound hypoventilation in horses, but a less severe cardiovascular depression at doses of 1-1.5 MAC compared with other inhalation anaesthetics (Steffey et al. 2005b). The reported cardiovascular effects are similar to those of isoflurane, although at concentrations above one MAC it may produce cardiovascular stimulation (Peck et al. 2008).

As previously stated, inhalant agents carried a higher risk of mortality compared with TIVA protocols (0.99 versus 0.31%) (Johnston et al. 2002). In that study most of the horses were anaesthetized with halothane. During the latter stages of data collection, isoflurane was licensed as an alternative inhalational anaesthetic agent. Experimental and clinical evidence suggests that isoflurane has a cardiovascular benefit over halothane (Grosenbaugh & Muir 1998; Rasis et al. 2000a; Blissitt et al. 2008), i.e. \dot{Q}_t is more depressed during halothane compared with isoflurane anaesthesia (Rasis et al. 2005). Isoflurane was also found to be safer in young horses and high risk cases (Johnston et al. 2004). During sevoflurane anaesthesia, haemodynamic and pulmonary indices were similar to those of isoflurane, with less pronounced decreases in \dot{Q}_t and systemic arterial pressure compared to halothane anaesthesia (Grosenbaugh & Muir 1998). Less pharmacological support (dobutamine) was needed during sevoflurane anaesthesia than in isoflurane anaesthetized horses, mainly because of less suppression of vasomotor tone (Driessen et al. 2006). Nevertheless, independently of the agent used, cardiovascular depression, hypoxaemia and hypoventilation are common problems associated with the use of volatile anaesthetics in horses and \dot{Q}_t , stroke volume and left ventricular work will decrease during anaesthesia, especially in dorsal recumbent horses (Gasthuys et al. 1991a).

Principles of treatment of cardiovascular depression

Cardiovascular depression and its consequences should be anticipated in order to reduce the complication rate in equine anaesthesia. Preventive measures include preoperative preparation of the horse, correcting abnormalities such as hypovolaemia, the use of an adequate padding and careful positioning once the horse is positioned on the surgical table, adequate monitoring, availability of equipment for artificial ventilation and last but not least, a firm reduction of anaesthetic time. Furthermore, cardiovascular function must be restored if required. Overall, three general principles are the fundamentals of the prevention and treatment of cardiovascular depression: high-volume fluid therapy, the use of cardiovascular stimulant drugs and reduction of anaesthetic depth.

Fluids can be administered in order to increase circulating volume and cardiovascular performance. The use of crystalloids as routine fluid therapy, colloids (Jones et al. 1997; Hallowell & Corley 2006) and hypertonic solutions (Dyson & Pascoe 1990; Schmall et al. 1990; Gasthuys et al. 1992) has been studied in anaesthetized horses. Cardiovascular stimulant drugs including adrenaline (Gaynor et al. 1992), dopamine (Gasthuys et al. 1991b; Lee et al. 1998), dopexamine (Muir 1992a, b; Lee et al. 1998), ephedrine (Hellyer et al. 1998) and dobutamine (Donaldson 1988; Gasthuys et al. 1991b; Lee et al. 1998; Rasis et al. 2000b) are also widely used during routine equine anaesthesia. The use of milrinone (Muir 1995), enoximone (Schauvliege et al. 2007, 2009), arginine vasopressin (Valverde et al. 2006), noradrenaline (Valverde et al. 2006), phenylephrine (Lee et al. 1998; Rasis et al. 2000c) or antimuscarinics (Teixeira Neto et al. 2004; Pimenta et al. 2011) has also been reported. However, these drugs are not always effective or free of side effects.

Reduction of the anaesthetic depth is another important method to treat cardiovascular depression. Volatile anaesthetics have only poor (if any) analgesic properties (Tomi et al. 1993; Petersen-Felix et al. 1995) and reducing anaesthetic depth is normally not possible when using only an inhalant agent for maintenance of anaesthesia, especially during painful surgical procedures. The use of locoregional techniques (Doherty et al. 1997; Haga et al. 2006) and/or systemically administered anaesthetics/analgesics (Muir & Sams 1992; Doherty & Frazier 1998; Bettschart-Wolfensberger et al. 2001) can reduce the need for volatile anaesthetics.

Balanced anaesthetic techniques in order to reduce the amount of inhalant agents

An ideal anaesthetic protocol includes a combination of different compounds, providing unconsciousness and analgesia, producing muscular relaxation and suppressing autonomic and somatic reflexes. This principle, the so-called ‘anociassociation’ was first described by George W. Crile (1910) who suggested the use of light general anaesthesia with local anaesthesia for blocking painful stimuli. The concept of ‘balanced anaesthesia’ was first introduced by John S. Lundy (1926) using different agents and techniques such as premedication, regional analgesia and general anaesthesia in order to achieve the different goals of an optimal anaesthetic procedure (analgesia, amnesia, muscle relaxation and reduction or elimination of autonomic reflexes while maintaining homeostasis). Currently, a single ideal anaesthetic drug is not available. Although some drugs have advantages in certain areas, they lack other important properties or may even produce side effects. More recently, the introduction of newer volatile agents and drugs with improved pharmacokinetics allowed this concept to reach new dimensions. Nowadays, the idea of balanced anaesthesia may differ in literature but it has been stated by Tonner (2005) that ‘a combination of anaesthetics will act synergistically with respect to the desired effects such as hypnosis or analgesia, but not with respect to side-effects’.

Balanced anaesthesia in horses is mainly applied for inhalation-based anaesthetic techniques, aiming to maintain a good intraoperative cardiopulmonary function followed by a calm, smooth and coordinated recovery (Bettschart-Wolfensberger & Larenza 2007). Although the ideal equine balanced anaesthetic technique is not available, different combinations have shown advantages and benefits. Local and regional anaesthetic techniques have been described (Doherty et al. 1997; Haga et al. 2006), while the concomitant use of inhalants and IV anaesthetics/analgesics has gained popularity (Muir & Sams 1992; Valverde et al. 2005; Ringer et al. 2007; Enderle et al. 2008).

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Section 1

Intravenous drugs used in combination with inhalation anaesthesia

Summary

All volatile anaesthetics depress cardiovascular function dose dependently. Drugs that reduce the requirements for volatile anaesthetics might improve cardiovascular function during anaesthesia. Different drugs can be administered systemically for this purpose. Because of its analgesic properties, lidocaine, a classic local anaesthetic drug, can be used to reduce the requirements for volatiles. However, ataxia resulting in uncontrollable recoveries may occur so the infusion should be discontinued thirty minutes before the end of anaesthesia. Ketamine, a dissociative agent, can also be infused at a low dose to obtain additional analgesia, amnesia and immobility, with minimal depression of the cardiovascular function reducing the requirements of the inhalant agents. Unwanted emergence reactions have been reported during the recovery period. Although the systemic use of opioids is supposed to provide a high level of perioperative analgesia, the use of these narcotics remains controversial in horses, mainly because of adverse effects including behavioral changes and ileus, without clear reductions in the minimal alveolar concentration (MAC) of the inhalants. Finally, α_2 -agonists provide sedation and analgesia in anaesthetized horses, considerably reducing the MAC, but may have a negative impact on cardiovascular function.

1.1. Lidocaine

Lidocaine is an amino amide local anaesthetic that has traditionally been used to provide local anaesthesia in man and animals but also for the treatment of premature ventricular contractions when administered systemically (Peck et al. 2008). The local anaesthetic properties are mainly induced by the blockade of sodium channels (Peck et al. 2008) although other mechanism of actions have been reported, some of these effects occurring at lower concentrations (Hollmann & Durieux 2000). Other sites of action include calcium (Xiong & Strichartz 1998) and potassium channels (Bräu et al. 1998) and M₁ muscarinic (Hollmann et al. 1999), glycine (Hara et al. 1995), gamma-aminobutyric acid (GABA)_A (Sugimoto et al. 2000), G protein-coupled (Hollmann et al. 2001) and N-methyl-D-aspartate (NMDA) receptors (Sugimoto et al. 2003).

In human medicine, the use of intravenous (IV) lidocaine for anaesthetic and analgesic purposes was first reported over sixty years ago (Gilbert et al. 1951; De Clive-Lowe et al. 1958) Its use decreased for over thirty years due to toxicity matters. Because local anaesthetics were shown to be efficient at blood concentrations lower than those considered to be toxic (Rimbäck et al. 1986, 1990), a renewed interest was formulated in the 1980s for new applications of IV lidocaine, such as the treatment of neuropathic pain (Kastrup et al. 1987; Ferrante et al. 1996) and the reduction of the duration of colonic stasis (Rimbäck et al. 1990). Additionally, IV lidocaine decreases postoperative pain (Koppert et al. 2004), has antihyperalgesic (Koppert et al. 1998) and anti-inflammatory properties (Hollmann & Durieux 2000), improves gastrointestinal function postoperatively (Groudine et al. 1998), facilitates rehabilitation (Kaba et al. 2007) and reduces the minimal alveolar concentration (MAC) of volatile agents (Himes et al. 1977).

Systemically administered lidocaine has recently gained popularity in equine anaesthetized patients as it produces anaesthetic-sparing (Doherty & Frazier 1998; Dziki et al. 2003), analgesic (Murrell et al. 2005; Robertson 2005) and anti-inflammatory effects (Nellgård et al. 1996; Cook et al. 2009). The mechanism by which lidocaine reduces the MAC of volatile anaesthetics may involve different receptor types, such as NMDA, GABA_A, acetylcholine and glycine (Zhang et al. 2007). Lidocaine dose dependently reduced the MAC of halothane in six experimental ponies (Doherty & Frazier 1998) receiving a loading dose (2.5 or 5 mg/kg) over five minutes followed by a CRI (50 or 100 µg/kg/min) for one hour. In a clinical study performed in twelve horses, Dziki et al. (2003) reported that IV administration of lidocaine at 2.5 mg/kg over ten minutes (fifteen minutes after induction)

followed by a CRI of 50 µg/kg/min during seventy five minutes resulted in a 25% reduction in isoflurane requirement, without negative effects on the cardiovascular system. Administration of IV lidocaine in colic horses (1.5 mg/kg bolus before surgery followed by a CRI 30 µg/kg/min) produced analgesia and dose dependent MAC sparing effects ranging from 20-25% with no significant cardiovascular or other side effects (Driessen 2005). More recently, the administration of a bolus of lidocaine (1.3 mg/kg) over fifteen minutes followed by 50 µg/kg/min CRI in eight experimental adult horses was shown to reduce the MAC of sevoflurane by 27% (Rezende et al. 2011). Nevertheless, under clinical circumstances, management of anaesthesia in horses receiving lidocaine (2 mg/kg over fifteen minutes plus CRI of 50 µg/kg/min) was more difficult and a higher expired fraction of isoflurane (F_E ISO) was required to maintain an appropriate, stable surgical plane of anaesthesia compared with those receiving medetomidine (Ringer et al. 2007).

The new concept of partial intravenous anaesthesia (PIVA) has been recently introduced in equine anaesthesia (Bettschart-Wolfensberger & Larenza 2007; Nannarone & Spadavecchia 2012). This principle has been defined as a form of balanced anaesthesia and implies the use of low concentrations of inhalation anaesthetics in combination with more than one injectable agent to reduce the cardiorespiratory depressant effects and to improve anaesthesia and anaesthetic stability. The co-administration of IV lidocaine and ketamine in horses was reported to produce an additive effect on the inhalant anaesthetic-sparing effects. This was first suggested by Enderle et al. (2008) and confirmed by Villalba et al. (2011), with MAC reductions of 40% and 49% respectively at different doses and rates. In a recent blinded clinical trial, the mean F_E ISO in anaesthetized horses undergoing elective surgery when receiving lidocaine and ketamine infusions was 1% (0.62-1.2%) and was further reduced to 0.65% (0.4-1.0%) when a medetomidine CRI was added (Kempchen et al. 2012).

With regard to antinociception, the mechanism whereby systemic lidocaine exerts an analgesic action has not been completely elucidated. Tanelian & MacIver (1991) suggested that the analgesia produced by lidocaine is the result of the suppression of tonic neural discharges in injured peripheral A-delta and C fibre nociceptors, although a direct action on spinal transmission in the spinal cord has also been proposed (Woolf & Wiessenfeld-Hallin 1985; Nagy & Woolf 1996; Koppert et al. 2000). It may also be possible that both peripheral and central actions contribute to the analgesic action of systemic lidocaine and that the predominant mechanism varies according to the nature of pain (Wallace et al. 1996). Low doses of systemic lidocaine have been used with good results for the treatment of severe cases of laminitis in equine patients (Malone & Graham 2002). Furthermore,

electroencephalographic findings have demonstrated that lidocaine provides antinociception contributing to additional analgesia during castration in ponies (Murrell et al. 2005). However, much less is understood about the action of lidocaine on the visceral pain. Indeed, lidocaine did not have a significant effect on the response to colorectal or duodenal distension in horses (Robertson et al. 2005), although it did inhibit, the cardiovascular responses to colorectal distension in rats, in a dose dependent manner (Ness 2000). Furthermore, lidocaine significantly increased the thermal threshold in horses (Robertson et al. 2005), which is in clear contrast with the findings in human volunteers, where systemic lidocaine had no effect on thermal thresholds (Wallace et al. 1997).

The molecular mechanisms involved in the anti-inflammatory effects are not well described, although it has been suggested that lidocaine has effects on cyclic adenosine monophosphate, G protein-coupled receptors, nicotinamide adenine dinucleotide, sodium-hydrogen antiporter and protein kinase C (Hollmann & Durieux 2000), reduces inducible nitric oxide synthase by suppression of nuclear factor κ B activation (Huang et al. 2006) and reduces the *ex vivo* production of interleukins IL-1 α and IL-6 (Yardeni et al. 2009). Systemic lidocaine has also been shown to provide other potential benefits in the equine, such as reduction in the incidence of postoperative ileus (POI) (Cohen et al. 2004), with desirable effects on intestinal motility (Brianceau et al. 2002) and resulting in a shorter hospitalization time in horses with POI (Malone et al. 2006). Furthermore, in horses with ischaemia-injured jejunum, lidocaine reduced the plasma prostaglandin E₂ metabolite concentration and mucosal cyclooxygenase-2 expression and ameliorated the flunixin-induced increase in neutrophil counts (Cook et al. 2009) and has been investigated for its potential therapeutic effects in models of endotoxemia in laboratory animals (Taniguchi et al. 1996; Schmidt et al. 1997).

Recovery from general anaesthesia is the most critical phase when anaesthetizing horses. No negative effects were noted by Dziki et al. (2003) during the recovery period in horses receiving a CRI of lidocaine throughout anaesthesia compared with a saline group. When a bolus of 1.5 mg/kg of lidocaine was administered just before surgery and the infusion of 30 μ g/kg/min stopped when the surgeon started to close the abdomen, horses with lidocaine did not show worse recoveries than those of the control group (Driessen 2005). In contrast, Valverde et al. (2005) described in a clinical study involving fifty four horses (2 mg/kg over fifteen minutes followed by 50 μ g/kg/min) higher degrees of ataxia and lower recovery qualities in horses receiving lidocaine until the end of the surgery. These authors recommended to discontinue the CRI thirty minutes before the end of the surgery. Ringer et

al. (2007) reported significantly better recoveries after the continuous infusion of medetomidine (3.5 µg/kg/hr) compared with lidocaine where both CRIs were stopped at the end of surgery and sedation was given prior to recovery. Furthermore, in a clinical study performed in twelve horses undergoing elective surgery, the addition of a medetomidine infusion (5 µg/kg/hr) to an infusion of lidocaine (2 mg/kg plus infusion at a rate of 50 µg/kg/min) improved the quality of recovery compared with lidocaine alone (Valverde et al. 2010a). In horses undergoing field castration, a bolus of lidocaine (3 mg/kg, IV) did not affect the recovery quality, although the overall recovery period was longer. Its use also did not reduce the needs for additional injectable anaesthesia during surgery (Sinclair & Valverde 2009).

In the equine, lidocaine is metabolized via the hepatic cytochrome P450 (CYP450) system into the active metabolites monoethylglycinexylidide and glycinexylidide, both lidocaine and the metabolites being excreted in the urine (Doherty & Frazier 1998). As it is highly metabolized by the liver and has a very short half-life (Engelking et al. 1987), it may be used intraoperatively as a bolus followed by a CRI. Lidocaine clearance is highly dependent on hepatic blood flow (Engelking et al. 1987) and general anaesthesia has a profound effect on serum lidocaine concentrations in horses, mainly due to a decrease in the volume of distribution and clearance of lidocaine (Feary et al. 2005). Moreover, other anaesthetic drugs metabolized via the CYP450 system may compete for binding sites and delay clearance (Doherty & Seddighi 2010).

Toxicosis should be considered when lidocaine is included in balanced anaesthetic protocols, especially because its neurological signs (weakness or ataxia) may be masked by anaesthesia. In the last decade, efforts have been made to determine the toxic blood levels for lidocaine in the horse. Meyer et al. (2001) demonstrated that lidocaine produced muscle fasciculations, tremors and ataxia in healthy awake horses at plasma levels between 1.85-4.53 µg/mL, substantially different from humans (1.56 ± 0.61 µg/mL) (Wallace et al. 1997) and dogs (8.21 ± 1.69 µg/mL) (Wilcke et al. 1983). Serum lidocaine concentrations ranged from 1-2 µg/mL in awake horses after abdominal surgery, receiving a loading dose of 1.3 mg/kg, followed by a CRI of 50 µg/kg/min. This resulted in a reduction in the volume of gastric reflux in horses with proximal enteritis and ileus postoperatively, while clinical signs of toxicosis were not observed (Malone et al. 1999). In patients undergoing elective procedures, Feary et al. (2005) showed that general anaesthesia with sevoflurane has a profound effect on lidocaine disposition in horses, and that lidocaine plasma levels were higher during anaesthesia than in awake horses (3.35 ± 0.60 and 1.85 ± 0.39 µg/mL respectively) after a

General introduction

loading dose of 1.3 mg/kg followed by a CRI of 50 µg/kg/hr. Although no clinical signs of toxicosis were observed, the authors speculated that general anaesthesia may mask neurologic manifestations of toxicosis. Lower doses compared to previous studies were recommended by Brianceau et al. (2002), who used a loading dose of 0.65 mg/kg during the first thirty minutes of general anaesthesia followed by a maintenance rate of 25 µg/kg/min in colic horses. Lidocaine had also favourable effects on jejunal distension and peritoneal fluid accumulation after abdominal surgery. The mean intraoperative lidocaine concentration was 1.06 ± 0.6 µg/mL, although in one horse intraoperative concentrations of 2.72 µg/mL were found. The authors attributed this variability mainly to individual differences in cardiac output (\dot{Q}_t). Horses experiencing pain may have a higher \dot{Q}_t , higher clearance of the drug and lower lidocaine serum concentrations. More compromised patients, with impaired cardiovascular function, will have a reduced liver blood flow and metabolism and higher lidocaine plasma levels.

In conclusion, lidocaine can be included in balanced anaesthetic protocols at different doses, providing intraoperative analgesia and reducing (dose dependently) the MAC of the volatile agent. However, potential side effects such as toxicosis should be taken into account, especially in cardiovascularly impaired patients. Furthermore, the infusion should be stopped at least thirty minutes before the end of anaesthesia to reduce the incidence of ataxia, improving the quality of the recoveries. An overview of the use of lidocaine in equine anaesthesia is provided in Table 1.

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Table 1: Different loading doses and infusion rates reported for the use of IV lidocaine in equine balanced anaesthesia.

Animals	Loading dose mg/kg	CRI $\mu\text{g}/\text{kg}/\text{min}$	Main findings	Reference
6 experimental ponies	2.5-5 over 5 mins	50-100	\downarrow MAC _{HALO} dose dependently	Doherty & Frazier 1998
28 colic horses	0.65 over 30 mins	25	Desiderable intestinal effects	Brianceau et al. 2002
12 healthy clinical horses	2.5 over 10 mins (15 mins after induction)	50	\downarrow MAC _{ISO} by 25% No bad recoveries	Dzikiti et al. 2003
50 colic horses	1.5 before start of surgery	30	\downarrow MAC _{ISO & SEVO} by 20-25% No worse recoveries	Driessen 2005
54 healthy clinical horses	2 over 15 mins	50	Affects degree of ataxia Stop CRI 30 mins before end of surgery	Valverde et al. 2005
16 experimental horses	1.3 over 15 mins	50	Anaesthesia influences lidocaine disposition	Feary et al. 2005
69 healthy clinical horses	2 over 15 mins	50	Maintenance easier and lower F _E ' _{ISO} with medetomidine	Ringer et al. 2007
8 experimental horses	1.3 over 15 mins	50	\downarrow MAC _{SEVO} by 27%	Rezende et al. 2011

CRI = constant rate infusion, MAC = minimum alveolar concentration, HALO = halothane, ISO = isoflurane, SEVO = sevoflurane, F_E' = expired fraction.

1.2. Ketamine

Ketamine is a drug discovered in 1962 and was introduced into human clinical anaesthesia in 1964 (Reich & Silvey 1989; Jansen 2000). It causes an anaesthetic trance-like state referred to as ‘dissociative anaesthesia’ (Domino et al. 1965), producing an electrophysiological dissociation between the thalamo-neocortical and limbic systems (Kohrs & Durieux 1998). This term has been used in humans who reported a feeling of being dissociated from their body and environment after administration of ketamine (Muir 2009). Although its role in human medicine has diminished with the introduction of newer IV anaesthetics, ketamine is still widely used outside the hospital for emergencies, disaster situations, and in third world countries as it is reliable for induction and maintenance of general anaesthesia, providing good surgical conditions with few side effects (Paix et al. 2005; Visser & Shug 2006). Moreover, its use at subanaesthetic doses is accepted in order to provide multimodal analgesia in patients with pain related to opioid tolerance but also for the treatment of acute severe, neuropathic, ischaemic, peripheral somatic, visceral, cancer or chronic post surgical pain (Menigaux et al. 2001; Petrenko et al. 2003; Correll et al. 2004; Visser & Schug 2006). Nonmedical use of ketamine has been unfortunately linked to the ‘dance culture’ (Jansen 2000).

Ketamine is a non-competitive antagonist of the NMDA receptor, which is a ligand gated calcium channel with glutamate as its major endogenous agonist (Kohrs & Durieux 1998). The NMDA receptors are of major importance for normal brain function and have a central role in learning, memory and the development of central nervous system (CNS) hyperactive states (Petrenko et al. 2003). Blockade of NMDA receptors enhances analgesia, but when exaggerated may result in memory impairment, excitation, dementia, ataxia and motor incoordination (Muir 2010). Interaction with NMDA receptors is responsible for general anaesthetic effects and analgesia (Visser & Schug 2006). However, although most of its effects (analgesic, amnesic and neuroprotective) are mediated via NMDA receptors (Kohrs & Durieux 1998; Chang et al. 2002), ketamine also interacts with non-NMDA glutamate, opioid, nicotinic, muscarinic and GABA_A receptors (Kohrs & Durieux 1998; Knobloch et al. 2006). The clinical use of NMDA antagonists at routine doses can be restricted mainly because of the psychomimetic side effects, ataxia and uncoordinated motor activity (Petrenko et al. 2003). These side effects are dose dependent and less common when using small, subanaesthetic doses (Himmelseher & Durieux 2005).

In contrast to the other drugs used for induction of anaesthesia, ketamine produces indirect cardiovascular stimulation with significant increases in blood pressure and heart rate (HR) (Zielmann et al. 1997). Additionally, it induces only a minimal respiratory depression with mild hypercapnia (Werner et al. 1997) and has been shown to antagonize the hypoventilation induced by alfentanil in man (Persson et al. 1999). In horses, IV doses of 2.2 mg/kg combined with xylazine provided excellent short term anaesthesia with stable cardiorespiratory function (Muir et al. 1977). Heart rate and mean arterial blood pressure (MAP) did not change when plasma ketamine concentration increased but \dot{Q}_t did significantly increase during ketamine infusion (Muir & Sams 1992). In contrast with these findings, Lankveld et al. (2006) reported significant increases in respiratory rate (RR), HR and arterial blood pressure during infusion of ketamine at different rates.

In equine practice, ketamine has been used for induction and maintenance of general anaesthesia for many years and has become a popular drug, especially when combined with an α_2 -agonist or centrally acting muscle relaxants (Muir et al. 1977; Butera et al. 1978; Luna et al. 1997; Hubbell et al. 2000). Nowadays, ketamine is also administered in horses in combination with other drugs to achieve multimodal analgesia for acute and chronic pain (Muir 2010). Moreover, it produces effective epidural analgesia (Gómez de Segura et al. 1998) and local anaesthetic effects (López-Sanromán et al. 2003a, b).

When administered IV in the equine, ketamine produces antinociceptive (Johnson et al. 1999; Knobloch et al. 2006; Peterbauer et al. 2008; Levionnois et al. 2010a) and anaesthetic effects (Bettschart-Wolfensberger et al. 1996; Mama et al. 2005). Up to now, the possible role in the treatment of equine endotoxaemia, remains controversial (Lankveld et al. 2005; Alcott et al. 2011). A 1.5 mg/kg/hr CRI of ketamine in healthy conscious horses showed that an infusion of ketamine can be safely administered for at least six hours (Lankveld et al. 2006). In contrast, Fielding et al. (2006) studied the effects of three different infusion rates (0.4, 0.8 and 1.6 mg/kg/hr) in adult conscious horses and clear signs of excitation after two hours of infusion of the the highest dose were observed. Ketamine also reduced the inhalant anaesthetic requirements up to 37% (related to its plasma concentration) in halothane anaesthetized horses (Muir & Sams 1992). Rates reported during equine balanced anaesthesia range between 0.5 and 3 mg/kg/hr (Muir 2010).

Ketamine is frequently included not only in total intravenous anaesthetic protocols (TIVA) with other drugs for the maintenance of general anaesthesia (Greene et al. 1986; Watkins et al. 1987; Flaherty et al. 1997; Umar et al. 2006; Hubbell et al. 2012) but also in multiple PIVA protocols (Spadavecchia et al. 2002; Yamashita et al. 2002; Kushiro et al.

2005; Enderle et al. 2008; Villalba et al. 2011; Kempchen et al. 2012; Nannarone & Spadavecchia 2012). Moreover, experimental studies have been performed in ponies in order to achieve predictable and stable blood drug concentrations, avoiding problems such as under- or overdosage by means of target-controlled infusions (TCIs) of ketamine (Knobloch et al. 2006; Levionnois et al. 2010b). Specific software allows to set a target drug concentration and adjusts the administration rate according to predicted drug concentrations in plasma, offering better control than CRIs (Hu et al. 2005; Levionnois et al. 2010b).

Ketamine is a racemic mixture of R- and S-enantiomers, the latter having approximately four times greater affinity for the active site of the NMDA receptor, resulting in an increase of the hypnotic properties (White et al. 1985; Oye et al. 1992). Currently, S-ketamine is only available in some European countries for veterinary use. Biotransformation of ketamine in the equine occurs by the hepatic and lung microsomes (Knobloch et al. 2006; Schmitz et al. 2008). Ketamine is extensively metabolized by the hepatic CYP450 to the active metabolite norketamine (S- or R-norketamine) via N-demethylation, which possesses a potency of one-third to one-fifth compared to the parent compound, but may be involved in the prolonged analgesic actions of ketamine (Kohrs & Durieux 1998). Norketamine forms hydroxynorketamine via hydroxylation (White et al. 1982), is later metabolized to dehydronorketamine (Lankveld et al. 2006) and is also eliminated by renal excretion (Sams & Pizzo 1987). Older studies reported that the elimination half-life of racemic mixture was approximately one hour (Kaka et al. 1979; Waterman et al. 1987). In contrast, S-ketamine was proven to have a higher clearance compared to the R-enantiomer not only during the administration of the racemic mixture but also when the two enantiomers were administered separately (Geisslinger et al. 1993; Ihmsen et al. 2001). In humans and horses, more recent studies suggest that ketamine pharmacokinetics are ‘context sensitive’ and differ depending on the dose, mode of administration (bolus or infusion) and mainly the duration of administration (White et al. 2006; Larenza et al. 2009a). *In vitro* studies described a slower elimination of S-ketamine in the presence of the R-enantiomer in human (Kharasch & Labroo 1992) and equine models (Schmitz et al. 2008). Clinical studies supported this assumption as elimination of S-ketamine was faster when given alone than when administered as part of a racemic mixture in standing ponies sedated with xylazine (Larenza et al. 2008) and in unsedated ponies (Larenza et al. 2009a). In contrast, the pharmacokinetics of racemic or S-ketamine in isoflurane anaesthetized ponies did not differ significantly (Larenza et al. 2007), mainly due to changes in hepatic and renal enzyme activities mediated by the different co-administered drugs.

As a consequence of its faster elimination, S-ketamine offers shorter recovery times than the racemate in humans (Engelhardt et al. 1998; Ihmsen et al. 2001). In ponies, S-ketamine (1.1 mg/kg, IV) produced a similar degree of immobility after a single injection and faster recoveries compared with the racemate (2.2 mg/kg, IV) (Larenza et al. 2008). When administered as a CRI in standing ponies at subanaesthetic doses, S-ketamine was considered to be a better option because of the suggested faster elimination (Larenza et al. 2009a). In an experimental trial involving ten horses undergoing elective arthroscopy, the recovery from anaesthesia was better in horses that were premedicated with xylazine and where anaesthesia was induced with S-ketamine (1.1 mg/kg, IV) and maintained with isoflurane and a CRI of S-ketamine (0.5 mg/kg/hr) compared to horses where the racemic mixture was used instead (Larenza et al. 2009b). Additionally, two clinical studies including horses for castration using TIVA, showed that horses receiving S-ketamine had quieter and more controlled recoveries compared to those where the classic ketamine was administered (Filzek et al. 2003; Rossetti et al. 2008). Moreover, Rossetti et al. (2008) reported minor excitatory effects during induction, with lower doses of other anaesthetic drugs for maintenance of anaesthesia when S-ketamine was administered. Unfortunately, it has been stated that S-ketamine may lack an antinociceptive effect as a result of rapid metabolism and lower plasma concentrations in the equine (Peterbauer et al. 2008).

Ketamine and its metabolites may produce excitatory side effects that can turn into fatal events in the recovery period in horses (Schatzmann & Girard 1984; Bettschart-Wolfensberger & Larenza 2007), mainly associated with the R-enantiomer (White et al. 1985; Filzek et al. 2003). When given at low doses in standing ponies, S-ketamine produced more ataxia and disorientation compared to the racemate, but these effects were of short duration (Peterbauer et al. 2008). Side effects in the recovery such as excitement or ataxia may occur when using ketamine as the sole agent for induction and maintenance of general anaesthesia (Bettschart-Wolfensberger et al. 1996). Furthermore, when infused in combination with other anaesthetics/analgesics, its adverse effects during the recovery phase may be prevented by reducing ketamine administration early enough before recovery (Knobloch et al. 2006). Prolonged infusions may lead to excessive norketamine formation and accumulation in fat and muscle (Knobloch et al. 2006), causing undesirable side effects. Consequently, IV boluses higher than 2 mg/kg or CRIs exceeding 1 mg/kg/hr should not be used in anaesthetics longer than 90-120 minutes while ketamine CRIs should be reduced in long procedures and/or stopped fifteen to twenty minutes before the recovery (Bettschart-Wolfensberger & Larenza 2007). Alternatively, an α_2 -agonist can be administered to reduce the incidence of

complications during the recovery phase (Santos et al. 2003). Furthermore, ketamine infusions may produce increases in gastrointestinal transit time and decreases in fecal output (Elfenbein et al. 2011).

In summary, low doses of IV racemic or S-ketamine may be useful in equine balanced anaesthetic protocols because of the demonstrated analgesic effects, the reduction of the anaesthetic requirements and the improvements of the cardiovascular haemodynamics. However, caution should be taken to prevent its undesirable excitatory side effects that may worsen the quality of the recoveries, mainly by avoiding high doses/rates and prolonged infusion times.

1.3. Opioids

Opium, extracted from the poppy seeds (*Papaver somniferum*) for thousand years, has been used in the treatment of cough and diarrhea and to relieve pain, while it may also produce euphoria (Kieffer 1999). The active ingredients of opium are alkaloid compounds, the so-called opioids, which possess analgesic (Dickenson 1991) and addictive (Koob 1992) properties. Morphine was the first opioid isolated from the poppy seeds in 1803 by Seturner, although its structure was not elucidated until 120 years later by Gulland & Robinson (1923) (Janecka et al. 2004). Today, in human medicine, morphine remains the reference opioid and is clinically used as an analgesic, despite a considerable number of side-effects including respiratory depression, nausea and vomiting, sedation and drowsiness, constipation, urinary retention or multifocal myoclonus (Schug et al. 1992; Inturrisi 2002). Morphine has been used in veterinary medicine since many years in order to produce analgesia, different degrees of sedation and reduction of the MAC of inhalant anaesthetics in dogs, rats, pigs, monkeys and cats (Murphy & Hug 1982; Lake et al. 1985; Steffey et al. 1994; Ilkiw et al. 2002; Muir et al. 2003). Apart from morphine, other synthetic opioids such as pethidine (Steffey et al. 1977), butorphanol (Murphy & Hug 1982; Ko et al. 2000), methadone (Credie et al. 2010; Ferreira et al. 2011), fentanyl (Moon et al. 1995; Hellyer et al. 2001; Reilly et al. 2013), alfentanil (Lake et al. 1985; Hall et al. 1987a; Ilkiw et al. 1997), remifentanil (Allweiler et al. 2007; Ferreira et al. 2009) or sufentanil (Hall et al. 1987b; Polis et al. 2004) have been widely employed systemically in veterinary medicine for the same reasons.

Three different types of opioid receptors have been identified and cloned, the μ , δ and κ receptors (Kieffer 1999; Janecka et al. 2004). The diversity of opioid receptors is further extended by the existence of several subtypes of opioid receptors (Smith & Lee 2003). It has

been described that μ -receptors mediate the most potent antinociceptive effects, but are also responsible for the development of dependence and adverse effects (Kieffer 1999; Janecka et al. 2004). Lower efficacy in mediating pain relief with a reduced addictive potential has been linked to δ -receptors (Janecka et al. 2004). The use of κ -receptor agonists may be restricted to peripheral tissues, mainly due to their potential dysphoric effects (Kieffer 1999). Moreover, interactions between receptors play a major role in opioid actions. Local, involving receptors of the same tissue, and nonlocal interactions, between receptors located in different tissues, have been described (Smith & Lee 2003). An ‘orphan’ (ORL) receptor has also been identified, which mainly mediates anti-opioid, rather than typical opioid effects (Fukuda et al. 1994; Mollereau et al. 1994). Opioid receptors are part of a large superfamily of membrane-bound receptors that are coupled to G-proteins (Smith & Lee 2003). Opioid receptor binding, via activation of different types of G-proteins, may inhibit adenylyl cyclase activity, activates receptor-operated potassium ion currents and suppresses voltage-gated calcium ion currents (Inturrisi 2002; Lamont & Mathews 2007).

The analgesic effects of the opioids are due to the direct inhibition of the ascending transmission of nociceptive information from the spinal cord dorsal horn, and due to activation of pain-control circuits that descend from the midbrain via the rostral ventromedial medulla to the spinal cord (Lamont & Mathews 2007). Opioids have also been reported to produce peripheral analgesia and anti-inflammatory effects (Stein et al. 2001). Opioid receptors are located in the joints of dogs (Keates et al. 1999), horses (Sheehy et al. 2001), rats (Nagasaka et al. 1996) and humans (Lawrence et al. 1992) as well. Different opioids have been used in humans and animal species in different ways to provide analgesia, i.e. also as anaesthetic adjuvants for inhalation anaesthetics, with the aim to reduce their MAC values.

The use of opioids in horses can be justified by their analgesic and sedative properties, but remains controversial due to their undesirable effects (Bennett & Steffey 2002; Clutton 2010). The use of morphine for pain relief in horses was first reported in 1898 (Guinard 1898) and its excitatory effects were described one year later (Guinard 1899). In 1917, the sedative effects of a low dose of morphine in horses were described (Milks 1917). Twenty years later, Amadon & Craigie (1937) described a ‘minimal analgesic dose’ at 0.2 mg/kg and a ‘minimal excitant dose’ at 0.5 mg/kg. Opioids have been associated with ‘excitement and unpredictable’ reactions in horses (Tobin 1981) and it has been suggested by Kamerling et al. (1989) that the behavioural and cardiovascular effects of morphine are stronger than its analgesic effects. When morphine was administered in ‘pain-free horses’ at a dose of 0.12 mg/kg, Muir et al. (1979) did not detect behavioural effects, although higher doses (0.66

mg/kg) stimulated locomotor activity for four to five hours (Kalpravidh et al. 1984). When fentanyl was administered by rapid IV injection, a sharp increase in locomotor activity was observed (Tobin et al. 1979). The different behavioural reactions to opioids compared with other species such as dogs may be related to the binding and distribution of opioid receptors in the brain, as the equine cerebral cortex is rich in μ -opioid receptors (Hellyer et al. 2003). More recent studies showed that the horse's cerebral cortex possesses a high concentration of high-affinity μ -receptors and lower concentration of δ - and κ - receptors, which resembles that of other species where opioid-induced CNS stimulation occurs (Thomasy et al. 2007), and differs markedly from the dog (Sharif et al. 1990) and human cerebral cortex (Pfeiffer et al. 1982). In horses, κ -receptor opioids seem to promote less locomotor and sympathetic stimulation than μ -opioids (Bennett & Steffey 2002).

With respect to their volatile anaesthetic sparing effects, opioids did not consistently alter the MAC in the equine. When morphine was administered as boluses at two different doses (low dose at 0.25 mg/kg and high dose 2.0 mg/kg), the change in MAC of isoflurane ranged from -20.2 to +28.3% and -18.9 to +56.2% after low and high doses respectively (Steffey et al. 2003). In contrast, when morphine was administered as an IV bolus (0.15 mg/kg) followed by a CRI (0.1 mg/kg/hr) in halothane anaesthetized horses undergoing elective surgical procedures, horses tended to receive fewer and lower doses of additional anaesthetic drugs, although this was not of statistical significance (Clark et al. 2005). In a study performed in ponies, Matthews & Lindsay (1990) did not find a statistically significant reduction of the MAC of halothane when administering two different IV doses of butorphanol, and the MAC was even increased in two ponies. Moreover, Doherty et al. (1997) reported in seven ponies that butorphanol did not significantly change the MAC of halothane, with an increase in three ponies, decrease in one and no effects in the other three ponies. When assessing the effects of three plasma concentrations of alfentanil, no significant changes in the MAC of halothane were observed in five horses (Pascoe et al. 1993). When fentanyl was administered IV to eight isoflurane anaesthetized horses at different doses and rates, the results suggested that this drug might consistently decrease anaesthetic requirements (Thomasy et al. 2006). However, in a more recent study, Knych et al. (2009) found no consistent changes in the MAC of isoflurane in eight horses. When combined with other drugs such as xylazine, morphine did not further reduce the MAC of halothane in six healthy horses (Bennett et al. 2004) and the addition of a CRI of morphine to a combination of two CRIs of ketamine and lidocaine did not further decrease the MAC of isoflurane (Villalba et al.

2011). However, the MAC sparing effects of a CRI of morphine alone have been not determined in the equine.

Behavioural effects associated with the use of opioids in horses may affect recovery. Steffey et al. (2003) noticed individuals recovering violently with signs of CNS excitement when high doses (2.0 mg/kg) were administered IV in a MAC study in six adult horses, whereas horses that received the low dose (0.25 mg/kg) recovered well, with little evidence of ataxia. After receiving IV fentanyl, one horse showed a violent recovery where the horse 'frenetically attempted to circle in both directions and fell down several times' (Thomasy et al. 2006). Moreover, in the study reported by Knych et al. (2009), undesirable and excitatory behaviours were observed after fentanyl administration. The authors concluded that the routine use of fentanyl is not supported. On the other hand, in a retrospective study involving eighty four healthy horses, Mircica et al. (2003) found that the recovery quality was better in horses receiving IV boluses of morphine (0.1 and 0.17 mg/kg) five to ten minutes after induction. The same findings were reported by Love et al. (2006) in horses undergoing anaesthesia for upper respiratory tract surgery with significantly better recoveries in horses receiving morphine at two different doses (0.1 and 0.2 mg/kg, IV). In another study, recoveries from general anaesthesia receiving morphine IV (0.15 mg/kg followed by a CRI of 0.1 mg/kg/hr) were better, with fewer attempts to attain sternal recumbency and standing and shorter times from the first recovery movement to the time at standing, compared to horses where morphine was not administered (Clark et al. 2008).

Other side effects should be taken into consideration when using opioids in the equine, such as risks of reduction of gastrointestinal motility and postoperative colic (Roger et al. 1985; Sellon et al. 2004; Boscan et al. 2006) or respiratory depression that could lead to a rapid increase in arterial partial pressure of carbon dioxide during general anaesthesia (Steffey et al. 2003). Although Steffey et al. (2003) showed undesirable residual CNS stimulating locomotor effects after administration of morphine, box-walking behaviour after general anaesthesia was either not observed (Love et al. 2006; Clark et al. 2008) or only present in one horse out of fifty one receiving morphine (Mircica et al. 2003). In an experimental study involving eight isoflurane anaesthetized horses testing different concentrations of fentanyl, two horses required active cooling by applying ethanol to the skin when their temperatures reached 38.2°C (Thomasy et al. 2006). Although unclear, morphine may have been involved in the development of postoperative pulmonary oedema in two horses (Kaartinen et al. 2010). The authors hypothesized that a relative fluid overloading during the prolonged anaesthetic

period was worsened by ‘morphine-induced reduction in urine production, further aggravated by potential morphine-induced changes in pulmonary permeability’.

Clinical evidence seems to suggest that opioids enhance the sedative and analgesic effects of α_2 -agonists. Morphine, methadone and butorphanol improved the sedative effects of α_2 -agonists, decreasing the responses to external stimuli, with butorphanol producing the most reliable response (Clarke & Paton 1988). Butorphanol (0.05 mg/kg, IV) reduced the response to imposed stimuli in horses sedated with romifidine, with no further cardiovascular changes than those induced by romifidine but an increased degree of respiratory depression (Clarke et al. 1991). The combination of butorphanol or levomethadone with detomidine increased the nociceptive threshold to somatic pain in horses, thereby prolonging the analgesic effect of detomidine (Schatzman et al. 2001). Butorphanol (0.05 mg/kg) and romifidine (0.1 mg/kg) IV provided better sedation than romifidine alone and, although the quality of sedation was significantly better than when romifidine was combined with morphine (0.1 mg/kg), the latter was concluded to be a suitable alternative (Corletto et al. 2005). In horses undergoing exploratory laparoscopy, the IV combination of medetomidine and morphine resulted in reliable sedation and stable cardiorespiratory function (Solano et al. 2009). Romifidine (0.1 mg/kg) and butorphanol (0.05 mg/kg) IV resulted in a longer duration of sedation and analgesia than romifidine or butorphanol alone (DeRossi et al. 2009). The addition of both types of drugs when performing equine balanced anaesthesia may enhance sedation and provide multimodal analgesia while avoiding the potential excitatory side effects linked to the use of opioids in horses.

In conclusion, opioids may be added when using IV balanced anaesthetic techniques in horses providing additional analgesia and sedation. However, their inconsistent MAC reduction, CNS stimulation, reduced gastrointestinal motility and other side effects may limit its use. Combination with other drugs such as α_2 -agonists may enhance their sedative and analgesic properties, providing a multimodal analgesic approach while reducing their potential excitatory effects.

General introduction

Table 2: Effects reported after IV administration of different opioids in horses undergoing general anaesthesia.

Drug	Animals	Bolus	Infusion rate	Effects	Reference
Mor	6 experimental horses	0.25 mg/kg	-	Good recoveries, MAC (-20.2 to +28.3%)	Steffey et al. 2003
Mor	"	2.0 mg/kg	-	Bad recoveries, MAC (-18.9 to +56.2)	"
Mor	84 healthy clinical horses	0.1-0.17 mg/kg	-	Better recoveries with Mor	Mircica et al. 2003
Mor	6 experimental horses	0.1-0.2 mg/kg	-	Mor does not alter Xyl sparing effect	Bennett et al. 2004
Mor	38 clinical horses	0.1-0.2 mg/kg	-	Better recoveries	Love et al. 2006
Mor	38 healthy clinical horses	0.15 mg/kg	0.1 mg/kg/hr	Fewer & lower extra anaesthetics	Clark et al. 2005
Mor	22 healthy clinical horses	0.15 mg/kg	0.1 mg/kg/hr	Less attempts & short times to recover	Clark et al. 2008
Butor	9 experimental ponies	0.022-0.044 mg/kg	-	No changes MAC _{HALO}	Matthews & Lindsay 1990
Butor	7 experimental ponies	0.05 mg/kg	-	No changes MAC _{HALO}	Doherty et al. 1997
Alfent	5 experimental horses	3 different targeted plasma concentrations		No changes MAC _{HALO}	Pascoe et al. 1993
Fent	8 experimental horses	0.3-3.0-4.7 µg/kg	0.4-3.5-6.8 µg/kg/hr	↓ MAC _{ISO} , 1 violent recovery	Thomasy et al. 2006
Fent	8 experimental horses	4.2-6.2-8.3 µg/kg	6-9-12 µg/kg/hr	No changes MAC _{ISO} , bad recoveries	Knych et al. 2009

Mor = morphine, Butor = butorphanol, Alfent = alfentanil, Fent = fentanyl, MAC = minimum alveolar concentration, Xyl = xylazine, HALO = halothane, ISO = isoflurane.

1.4. Alpha₂-adrenoceptor agonists

Clonidine, the first α_2 -agonist, was synthesized at the beginning of the 1960s. This drug was used as a nasal decongestant, further developed as an antihypertensive agent and finally used for alleviation of the symptoms of opiate withdrawal (Stähle 2000). Xylazine was initially also commercialized in 1962 as an antihypertensive. Despite, or in fact because of its potent sedative side effects in humans (Kästner 2006), its use in veterinary medicine became popular (Rosenberger et al. 1968; Clarke & Hall 1969; Müller et al. 1969; Kerr et al. 1972). In the 1980s, different α_2 -agonists were developed such as detomidine (Virtanen et al. 1985; Lowe & Hilfiger 1986; Jöchle & Hamm 1986), medetomidine (Savola et al. 1986; Stenberg et al. 1987; Virtanen 1988), dexmedetomidine (Kallio et al. 1989) and romifidine (Gasthuys et al. 1990; England et al. 1992). Currently, xylazine, detomidine and romifidine are approved for use in horses in Europe. Medetomidine and dexmedetomidine are licensed for small animals only, although both drugs have been studied in horses (Bryant et al. 1991; Bettschart-Wolfensberger et al. 1999, 2005).

Adrenoceptors are situated on the cell membrane sites where noradrenaline and adrenaline act as important neurotransmitters in the peripheral and CNS. It was first observed by Dale (1905) that the pressor effect of adrenaline was reversed by ergotoxine, but it was not until 1948 that Ahlquist proposed the classification of adrenoceptors into α and β (Ahlquist 1948). Differentiation between α_1 receptors, which mediate the responses in the effector organs (i.e. heart, lung, liver, arteries) and α_2 , located presynaptically to regulate the release of the neurotransmitter, but also present postsynaptically, was suggested by Langer (1974) and later accepted by Berthelsen & Pettinger (1977). Molecular cloning technology and the development of more selective drugs revealed three α_2 -adrenoceptor subtypes ($\alpha_{2a/d}$, α_{2b} , α_{2c}) (Bylund 1994; Guimarães & Moura 2001). The division between α_1 and α_2 -adrenoceptors is made depending on their selectivity to specific agonist and antagonist agents (England & Clarke 1996). Clonidine, xylazine, romifidine, detomidine, medetomidine and dexmedetomidine are agonists whilst atipamezole and yohimbine are the classic antagonists (Scheinin & Macdonald 1989).

The α_2 -adrenoceptors are transmembrane receptors coupled to the inhibitory heterotrimeric GTP-binding protein inhibiting the activity of adenylyl cyclase (Cotecchia et al. 1990; Wise et al. 1997; Khan et al. 1999) and the opening of voltage-gated calcium channels (Cotecchia et al. 1990) while activating potassium channels (Surprenant et al. 1992). Presynaptic α_2 -adrenoceptors in sympathetic nerve endings and noradrenergic neurons in the

CNS inhibit the release of noradrenaline (Langer 1980). Postsynaptic α_2 -adrenoceptors are present in the liver, pancreas, platelets, kidney, adipose tissue and the eye (Khan et al. 1999). The locus coeruleus of the brain contains a high density of α_2 -adrenoceptors (Unnerstall et al. 1984) mediating the sedative action by α_{2a} -adrenoceptors (Scheinin & Schwinn 1992). High densities of α_{2a} -adrenoceptors are also present in the descending pathways within the spinal column, modulating nociceptive stimuli and interactions with opioids (Fairbanks et al. 2009). Furthermore, the α_2 -adrenoceptors are densely distributed in the dorsal motor nucleus of the vagus and nucleus tractus solitarius (Robertson & Leslie 1985), activating the cardiac vagal nerve through the action on those brainstem areas (Kawada et al. 2012), and in the superficial laminae and substantia gelatinosa of the dorsal horn (Nicholas et al. 1993). The dorsal horn of the spinal cord contains α_{2a} -adrenoceptors, while the primary sensory neurons contain both α_{2a} and α_{2c} (Murrell & Hellebrekers 2005). Centrally, the subtype α_{2b} has been found scarcely in the thalamic region in rats (Scheinin et al. 1994), although these receptors have been involved in modulating nitrous oxide mediated nociception in mice together with the α_{2a} -adrenoceptors (Guo et al. 1999; Sawamura et al. 2000). In vascular smooth muscle the α_{2b} -subtypes mediate peripheral hypertension after α_2 -agonist administration (Link et al. 1996). The α_{2c} -receptors influence several complex memory and behavioural functions (Björklund et al. 1999; Scheinin et al. 2001).

Alpha₂-agonists are potent sedatives with good analgesic properties which are used frequently in combination with other drugs during anaesthesia in horses. Side effects include bradycardia, arrhythmias, decreases in \dot{Q}_t and increases in systemic vascular resistance, respiratory depression, transient decreases in arterial partial pressure of oxygen and ataxia (England & Clarke 1996; Yamashita et al. 2000), especially after bolus administration. Reported sedative effects of α_2 -agonists in horses include decreased awareness, ptosis of the head, lower lip and eyelids, ataxia and a wide stance (England & Clarke 1996; Valverde 2010b), all of which are mediated through α_{2a} -receptors (Knaus et al. 2007). These sedative effects are related to their spinal and supraspinal actions, demonstrated in visceral pain models (Pipi & Lumb 1979; Muir & Robertson 1985; Kohn & Muir 1988; Elfenbein et al. 2009). Intestinal motility has been shown to decrease in horses after IV administration of different α_2 -agonists (Adams et al. 1984; Freeman & England 2001). On the other hand, as suggested by Valverde (2010b), α_2 -agonists could produce beneficial effects on the stomach as shown in rats, mediated by α_{2b} -receptors, inducing a gastroprotective effect by regulating gastric acid secretion and inhibiting chemically (non-steroidal anti-inflammatory drugs) and

physically (stress) induced gastric mucosal lesions (Gyires et al. 2000, 2009; Fülöp et al. 2005). Furthermore, these drugs are known to increase urine output in awake (Thurmon et al. 1984) and anaesthetized healthy equids (Tranquilli et al. 1984; Steffey & Pascoe 2002), mainly due to hyperglycaemia from hypoinsulinaemia (Gasthuys et al. 1986, 1987) mediated through α_{2a} and α_{2c} -receptors (Peterhoff et al. 2003) and additionally due to a reduced arginine vasopressin secretion (Alexander & Irvine 2000). In patients receiving α_2 -agonists during general anaesthesia, the placement of a urinary catheter is justified to avoid excessive bladder distension but also to monitor urinary output (Bettschart-Wolfensberger & Larenza 2007; Valverde 2010b).

The use of IV α_2 -agonists in balanced anaesthetic techniques has become more and more popular over the last years, mainly to reduce the MAC of inhalation anaesthetic agents and to provide sedation and analgesia perioperatively. Furthermore, their use is extended as their administration after inhalation anaesthesia improved the quality of recovery without producing significant cardiorespiratory effects (Santos et al. 2003).

Xylazine, the least selective α_2 -agonist [selectivity ratio (α_2/α_1) 160:1], has been used widely and successfully as a premedicant in the horse (England & Clarke 1996). Under experimental conditions, isoflurane MAC was reduced by 25 and 34% after administration of IV xylazine at 0.5 and 1.0 mg/kg respectively (Steffey et al. 2000) and halothane MAC by 20% after a bolus of 0.5 mg/kg (Bennett et al. 2004). Up to date, only one abstract has been reported using xylazine as a CRI (1 mg/kg/hr after 0.7-0.8 mg/kg) in isoflurane anaesthetized horses, resulting in pronounced reduction of blood pressure support and anaesthetic requirements compared to isoflurane alone (0.95 ± 0.07 versus 1.16 ± 0.13) (Pöppel et al. 2012).

A CRI of detomidine (260:1), administered with a TCI device to achieve a plasma level of 25 ng/mL (10.8 μ g/kg/hr), reduced the MAC of halothane by 33% in horses (Dunlop et al. 1991). Based on these results, the effects of a CRI of detomidine were investigated in nine halothane anaesthetized horses undergoing neurectomy (Wagner et al. 1992). One group consisted of five horses receiving a detomidine CRI to accomplish a plasma concentration of 25 ng/mL during halothane anaesthesia at an F_E ISO of 1.1%. In the remaining four horses, anaesthesia was maintained using 1.5 % F_E HALO. Both protocols should result in an equivalent depth of anaesthesia according to the previous preliminary trials by Dunlop et al. (1991). Apart from a significantly lower HR in the detomidine group, no other changes were reported in cardiovascular function and recovery parameters. A recent blinded clinical study

using a detomidine bolus (10 µg/kg) followed by a CRI (5 µg/kg/hr) was performed by our research group in twenty adult healthy horses undergoing elective surgery (Schauvliege et al. 2011). Typical cardiovascular effects of α_2 -agonists were observed, with no effect on the isoflurane requirements, recovery duration or recovery quality.

Recently, the pharmacokinetic profile and pharmacodynamic effects of romifidine (340:1) in the horse have been described (Wojtasiak-Wypart et al. 2012). Its use as a CRI for balanced anaesthesia was first reported by Kuhn et al. (2004) in a double-blinded clinical study of twenty isoflurane-anaesthetized horses undergoing elective surgery. After IV premedication with 80 µg/kg of romifidine, a CRI of 18 µg/kg/hr was continued. Isoflurane requirements were reduced significantly, with beneficial effects on the cardiovascular and pulmonary parameters. Unfortunately, the effect of mechanical ventilation was not taken into account, two different anaesthetists were involved and a description of the recoveries was lacking. In contrast, Devisscher et al. (2010) failed to detect inhalation sparing effects in a blinded clinical study involving thirty isoflurane anaesthetized horses undergoing routine arthroscopy when using the same loading dose followed by a CRI at 40 µg/kg/hr. This protocol did not affect cardiovascular function or recovery quality.

Although not licensed for horses, medetomidine (1620:1) has been widely studied in the equine. It has been used as a sedative/analgesic for restraining horses and as premedication prior to general anaesthesia (Bryant et al. 1991; England & Clarke 1996), with cardiovascular effects of short duration (Bryant et al. 1996; Yamashita et al. 2000). The pharmacokinetic and pharmacodynamic properties of medetomidine have been studied in ponies when administering an IV bolus of 7 µg/kg followed by a CRI of 3.5 µg/kg/hr (Bettschart-Wolfensberger et al. 1999) and in horses after an IV bolus of 10 µg/kg (Grimsrud et al. 2012). Properties such as high clearance and short half-life make the drug suitable for continuous infusion in the horses (Bettschart-Wolfensberger et al. 1999). Identical dosages as described by Bettschart-Wolfensberger et al. (1999) reduced the MAC of desflurane in an experimental trial involving seven healthy ponies by 28% compared to previously reported MAC values in literature (Bettschart-Wolfensberger et al. 2001). Clinical studies performed by the same group of researchers using the same protocol, found a reduction in the MAC of isoflurane of approximately 20% (Neges et al. 2003). In a retrospective study of Kalchofner et al. (2006) this protocol appeared to be suitable in horses undergoing routine surgery, with well maintained cardiopulmonary function and an incidence of hypotension and hypoxaemia comparable to other anaesthetic regimes. The latter study also described the 'lighter' appearance of horses under anaesthesia when medetomidine is infused compared with

traditional inhalant protocols and reported good and calm recoveries. When comparing this protocol with a lidocaine infusion, MAP tended to be higher and the surgical plane of anaesthesia was better maintained in horses receiving medetomidine, with less need of additional ketamine and thiopental (Ringer et al. 2007). Even more, the combination of an intraoperative CRI of medetomidine and lidocaine did not affect the cardiovascular function of isoflurane anaesthetized horses but improved the quality of the recovery when compared with lidocaine alone (Valverde et al. 2010a). In a recent study, the addition of a CRI of medetomidine to horses also receiving CRIs of lidocaine and ketamine reduced the $F_{E'}\text{ISO}$ from 1 to 0.65% (Kempchen et al. 2012).

To summarize, the α_2 -agonists used in combination with volatile agents reduced the MAC of these agents and provided extra sedation and analgesia, with ease of maintenance of anaesthesia and better recovery qualities. However, their impact on cardiovascular function should be considered. It is worth mentioning that all the studies reporting the use of α_2 -agonists were performed in healthy horses. The use of these drugs in compromised (colic) horses remains controversial. A list with the different studies using α_2 -agonists in equine balanced anaesthesia is provided in Table 3.

The cardiopulmonary effects and pharmacokinetics of the newest α_2 -agonist dexmedetomidine have been studied in ponies after IV administration (Bettschart-Wolfensberger et al. 2005). With similar cardiopulmonary effects to those reported by other α_2 -agonists, this drug was defined as a rapidly redistributed and short-acting sedative drug in horses, useful for CRIs protocols. Moreover, the administration of the active enantiomer of the racemic mixture alone may have some cardiovascular and analgesic benefits, being slightly more potent and predictable than medetomidine (Kuusela et al. 2001; Granholm et al. 2007).

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Table 3: Different loading doses and infusion rates reported for the use of IV α_2 -agonists in equine balanced anaesthesia.

Drug	Animals	Loading doses	Infusion rates	Main findings	Reference
Xyl	51 healthy clinical horses	0.7-0.8 mg/kg	1.1 mg/kg/hr	Pronounced ISO reduction	Pöppel et al. 2012
Det		Plasma concentration 25 ng/mL		↓MAC _{HALO} by 33%	Dunlop et al. 1991
Det	9 healthy horses	10.8 µg/kg/hr to achieve 25 ng/mL		↓MAC _{HALO}	Wagner et al. 1992
Det	20 healthy clinical horses	10 µg/kg	5 µg/kg/hr	No sparing ISO effects	Schauvliege et al. 2011
Rom	20 healthy clinical horses	80 µg/kg	18 µg/kg/hr	Significant reduction ISO	Kuhn et al. 2004
Rom	30 healthy clinical horses	80 µg/kg	40 µg/kg/hr	No sparing ISO effects	Devisscher et al. 2010
Med	5 experimental ponies	7 µg/kg	3.5 µg/kg/hr	Suitable for infusion	Bettschart-Wolfensberger et al. 1999
Med	7 experimental ponies	7 µg/kg	3.5 µg/kg/hr	↓MAC _{DESF} by 28%	Bettschart-Wolfensberger et al. 2001
Med	40 healthy clinical horses	7 µg/kg	3.5 µg/kg/hr	↓MAC _{ISO} by 20%	Neges et al. 2003
Med	69 clinical horses	7 µg/kg	3.5 µg/kg/hr	Maintenance easier and ↓ F _{E'} _{ISO} than lidocaine	Ringer et al. 2007

Xyl = xylazine, Det = detomidine, Rom = romifidine, Med = medetomidine, MAC = minimum alveolar concentration, HALO = halothane, ISO = isoflurane, DESF = desflurane, F_{E'} = expired fraction.

Conclusions

Several drugs and their combinations can be administered systemically in order to improve cardiovascular function and recovery qualities and to provide a multimodal analgesic approach, although their advantages and disadvantages should be evaluated. The use of the different protocols reviewed here requires some clinical experience in order to avoid intraoperative and postoperative complications, such as inadequate depth of anaesthesia and analgesia, toxicity, poor recovery qualities or postoperative complications. In the search for the ideal agent, previous results in ponies and other species pointed out dexmedetomidine as an attractive alternative for equine balanced anaesthetic protocols. An overview of data and dosages of different combination of drugs used for CRIs is provided in Table 4.

General introduction

Table 4: Different loading doses and infusion rates reported for use of IV combinations of drugs in equine balanced anaesthesia.

Drugs	Animals	Loading doses	Infusion rates	Main findings	Reference
Lid/Ket	40 healthy clinical horses	Lid: 1.5 mg/kg (10 mins) Ket: 3 mg/kg	Lid: 40 µg/kg/min Ket: 3.6 mg/kg/hr	↓ MAC _{ISO} by 40%	Enderle et al. 2008
Lid/Ket	6 experimental horses	Lid: 2 mg/kg (10 mins) Ket: 3 mg/kg	Lid: 50 µg/kg/min Ket: 3 mg/kg/hr	↓ MAC _{ISO} by 49%	Villalba et al. 2011
Lid/Ket/Mor	"	Lid/Ket as above Mor: 0.15 mg/kg	Lid/Ket as above Mor: 0.1 mg/kg/hr	↓ MAC _{ISO} by 53%	"
Lid/Ket	40 healthy clinical horses	Lid: 1.5 mg/kg (10 mins) Ket: 3 mg/kg	Lid: 33 µg/kg/min Ket: 2 mg/kg/hr	F _{E'ISO} 1% (0.62-1.2%)	Kempchen et al. 2012
Lid/Ket/Med	"	Lid/Ket as above	Lid/Ket as above Med: 3.6 µg/kg/hr	F _{E'ISO} 0.65% (0.4-1.0%)	"
Lid/Med	12 healthy clinical horses	Lid: 2 mg/kg (10 mins)	Lid: 50 µg/kg/min Med: 5 µg/kg/hr	Better recoveries than lidocaine alone	Valverde et al. 2010a

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Drugs	Animals	Loading doses	Infusion rates	Main findings	Reference
Gu/Ket	28 healthy clinical horses	Gu: 60 mg/kg Ket: 2.2 mg/kg	Gu: 0.3-1 mg/kg/min Ket: 0.9-2.4 mg/kg/hr	↓ $F_{E'}^{HALO}$ from 1.24% to 0.61%	Spadavecchia et al. 2002
Gu/Ket/Med	40 healthy horses	Ket: 2.2 mg/kg	Gu: 0.4 mg/kg/min Ket: 1 mg/kg/hr Med: 1.2 µg/kg/hr	↓ MAC_{SEVO} by 38%	Yamashita et al. 2002
Gu/Ket	45 healthy clinical horses	Gu: 50 mg/kg Ket: 2.2 mg/kg	Gu: 1 mg/kg/min Ket: 2.4 mg/kg/hr	Adequate anaesthesia at 0.75 MAC_{ISO}	Nannarone & Spadavecchia 2012
Rom/Ket	45 healthy clinical horses	Rom: 50 µg/kg Ket: 2.2 mg/kg	Rom: 24 µg/kg/hr Ket: 2.4 mg/kg/hr	Adequate anaesthesia at 0.75 MAC_{ISO}	Nannarone & Spadavecchia 2012
Mdz/Ket/Med	6 experimental horses	Mdz: 0.04 mg/kg Ket: 2.5 mg/kg Med: 5 µg/kg	Mdz: 0.02 mg/kg/hr Ket: 1 mg/kg/hr Med: 1.25 µg/kg/hr	Adequate anaesthesia at 0.74 MAC_{SEVO}	Kushiro et al. 2005

Lid = lidocaine, Ket = ketamine, Mor = morphine, Med = medetomidine, Gu = guaifenesin, Rom = romifidine, Mdz = midazolam, HALO = halothane, ISO = isoflurane, SEVO = sevoflurane, MAC = minimum alveolar concentration, $F_{E'}$ = expired fraction.

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Section 2

The use of dexmedetomidine for balanced anaesthesia in horses

Summary

Dexmedetomidine, the most selective α_2 -agonist, is a drug with a beneficial pharmacological profile (short half-life and rapid redistribution) that produces sedation, anxiolysis and analgesia. This α_2 -agonist has been used in human and veterinary medicine for years, more specifically in ‘balanced anaesthesia’ reducing the use of volatile anaesthetics. In the equine, only the pharmacokinetics and cardiopulmonary effects after administration of an intravenous bolus in ponies have been described. Dexmedetomidine was proven to be safe for sedation. Although the kinetics suggest its use as a continuous infusion, this had not been investigated in depth in anaesthetized equines at the beginning of this PhD.

2.1. History of dexmedetomidine

Medetomidine, a racemic mixture of two individual isomers (Vickery et al. 1988; Savola & Virtanen 1991) has been marketed as Domitor® (Orion Corporation, Espoo, Finland) for use in dogs and cats since 1987. Dexmedetomidine is the pharmacologically active optical enantiomer (Savola & Virtanen 1991), being entirely responsible for the sedative, analgesic and dose dependent anaesthetic sparing effects (Segal et al. 1988; Ansah et al. 1998; Kuusela et al. 2000). Reports have suggested that at equivalent concentrations, dexmedetomidine may be less likely to cause drug interactions compared to the racemate (Kharasch et al. 1992). Moreover, its use may offer advantages over medetomidine (Ansah et al. 1998; Kuusela et al. 2000), being slightly more potent (Savola & Virtanen 1991; Kuusela et al. 2000). The European Commission granted a marketing authorization for Dexdomitor® (Orion Corporation, Espoo, Finland) in 2002, which was renewed in 2007. It is indicated for use as a sedative and analgesic in dogs and cats to facilitate clinical examinations, clinical procedures, minor surgical procedures and as a pre-anaesthetic to general anaesthesia.

Dexmedetomidine (Precedex®, Hospira, Inc., Lake Forest, IL) was also approved for use in humans in the US specifically as a continuous infusion up to one day for sedation and analgesia in the intensive care unit (ICU). Afterwards, Dexdor® (Orion Corporation, Espoo, Finland) was licensed in 2011 in all European Union member states for use as a constant rate infusion (CRI) for sedation of adult ICU patients. Recent reports showed safety outcomes in patients receiving this α_2 -agonist over a long period of time (> twenty four hours) (Abuhasna et al. 2012).

2.2. Mechanism of action of dexmedetomidine

Dexmedetomidine is a potent α_2 -agonist with a high $\alpha_2:\alpha_1$ selectivity (approximately 1600:1) (Kamibayashi & Maze 2000). By virtue of this potency, it is considered to be a full α_2 -agonist, allowing the use of relatively high doses without the unwanted vascular effects resulting from stimulation of α_1 -adrenoceptors (Ebert et al. 2000). Even more, activation of these receptors may induce arousal, restlessness, increased locomotor activity and vigilance, antagonizing hypnosis (Monti 1982; Guo et al. 1991; Puumala et al. 1997). The α_2 -agonists produce their effects after binding to the α_2 -adrenoceptors

(Figure 1). As described in Section 1, α_{2a} -adrenoceptors in the brain are responsible for the antinociceptive, sedative, sympatholytic, hypothermic and behavioural responses (Paris & Tonner 2005). The α_{2b} -subtype mediates vasoconstriction and the short-term hypertensive response (Link et al. 1996; Kamibayashi & Maze 2000), while α_{2c} -adrenoceptors affect memory and behaviour and induce hypothermia (Scheinin et al. 2001). Its effects are effectively reversed by atipamezole, a selective α_2 -antagonist (Scheinin et al. 1998).

Dexmedetomidine also combines with the imidazoline receptors I1 (brain) and I2 (brain, kidney and pancreas), by recognizing its imidazoline-ring structure (Hieble & Ruffolo 1995; Khan et al. 1999). Central hypotensive effects are suggested to be attributed to the I1 receptors in the ventrolateral medulla (Bousquet et al. 1984; Ernsberger et al. 1990; Kamisaki et al. 1990). Moreover, I1 receptors produce central inhibition of catecholamine-induced dysrhythmias (Kamibayashi et al. 1995; Mammoto et al. 1996), with a low influence on sedation (Prichard & Graham 2000). Although not completely understood, I1 receptors are thought to be G-protein linked (Khan et al. 1999). The role of peripheral imidazoline receptors remains controversial. Imidazoline 1 receptors in the carotid bodies may act with opposite effects to those of peripheral α_2 -adrenoceptors (Ernsberger et al. 1998). Peripheral I2 receptors are found in mitochondrial membranes and their mechanism of action is not related to G-proteins (Regunathan et al. 1991a, b, 1993).

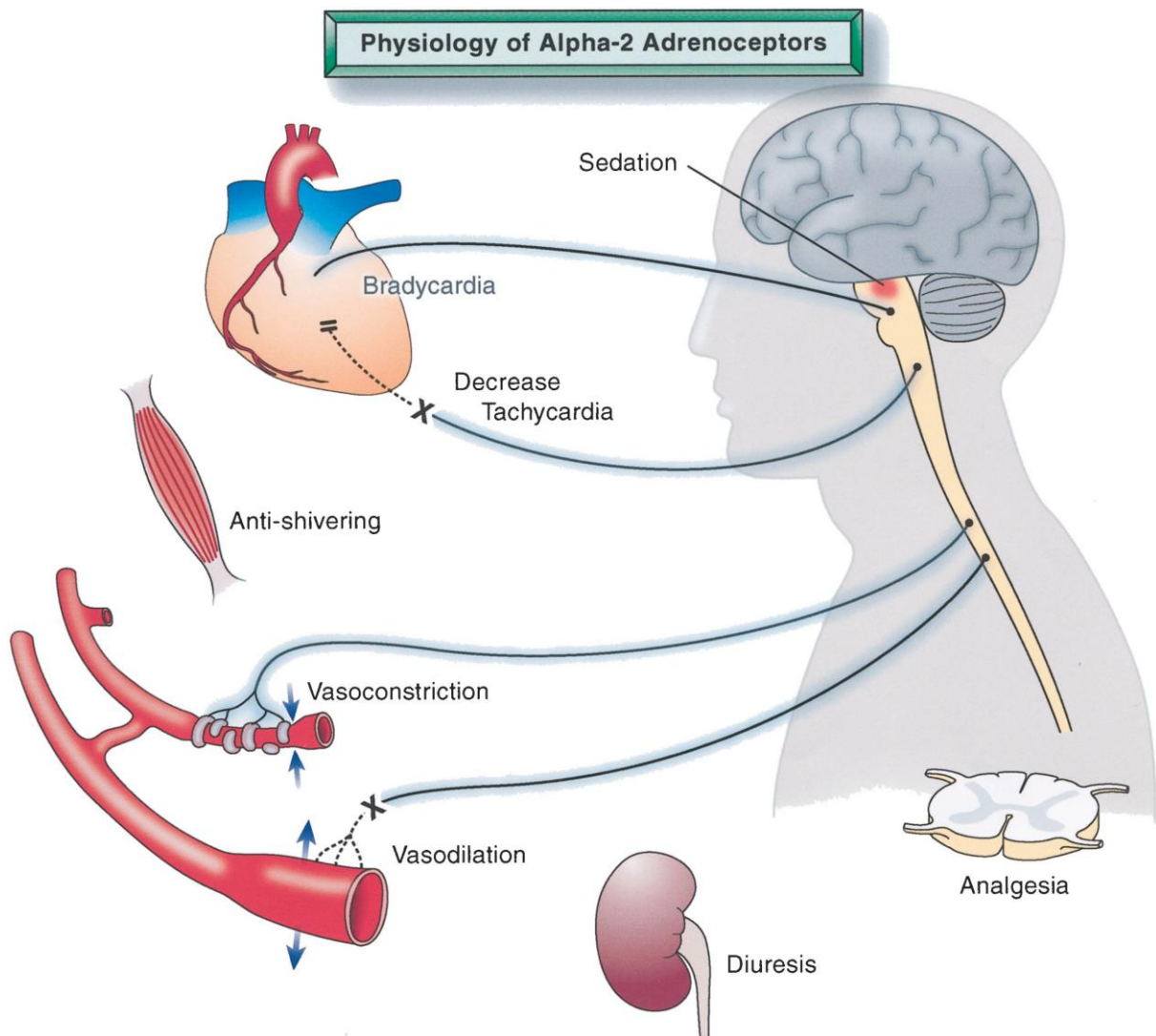


Figure 1: Possible responses mediated by α_2 -adrenoceptors. Adapted from Kamibayashi & Maze 2000.

2.3. Pharmacokinetics of dexmedetomidine

The metabolism of medetomidine has been studied in rats. It consists of hydroxylation with further glucuronidation or oxidation to carboxylic acid and production of eight metabolites (Salonen & Eloranta 1990). Metabolism via the lungs and the kidneys has also been described (Salonen 1991). Dexmedetomidine may reduce its own clearance and elimination rate dose dependently via its haemodynamic effects (Salonen et al. 1995; Kuusela et al. 2000; Escobar et al. 2012; Pypendop et al. 2013), most likely because liver blood flow decreases after administration of this drug (Lawrence et al. 1996a). In humans, the effect of dexmedetomidine on cardiac output

(\dot{Q}_t) influenced the pharmacokinetics of dexmedetomidine (Dutta et al. 2000). In contrast, studies in dogs suggested hepatic biotransformation to be the rate-limiting factor in the metabolic clearance rather than the degree of liver perfusion. Low rates of biotransformation in canine hepatocytes have been described for dexmedetomidine and its racemate (Kaivosaaari et al. 2002; Duhamel et al. 2010).

Kuusela et al. (2000) studied the pharmacokinetics of dexmedetomidine, levomedetomidine and the racemate in dogs. Equipotent doses of medetomidine and dexmedetomidine showed similar clearance, and a trend for the steady state volume of distribution (V_{ss}) and terminal half-life ($t_{1/2}$) to be lower after dexmedetomidine administration. Clearance and V_{ss} were significantly higher after levomedetomidine administration, with tendency of the $t_{1/2}$ to be lower. The authors concluded that dexmedetomidine may offer benefits over the racemic mixture, as the effects of the active enantiomer are easier to predict. While some discrepancies in the pharmacokinetics between both enantiomers could be explained by different rates of their hepatic biotransformation, equipotent doses of dexmedetomidine and its racemate still produced comparable plasma concentrations in dogs (Kuusela et al. 2000).

2.4. Clinical effects of dexmedetomidine

2.4.1. Sedation and analgesia

The sedative properties of α_2 -agonists are mediated via the α_{2a} -adrenoceptors, with the locus coeruleus (LC) as the main site of action (Correa-Sales et al. 1992; Mizobe et al. 1996). Dexmedetomidine has been successfully used in humans for sedation in the ICU (Venn et al. 1999; Panzer et al. 2009). Its analgesic effects are produced at the level of the spinal cord and supraspinal sites. Its main central antinociceptive action is mediated via spinal α_2 -adrenoceptors in the substantia gelatinosa within the dorsal horn (Howe et al. 1983; Kuraishi et al. 1985; Sullivan et al. 1992). A supraspinal component via the LC has also been suggested (Pertovaara et al. 1991; Guo et al. 1996), but this remains controversial (Hämäläinen & Pertovaara 1995). Studies with medetomidine in humans suggested that analgesia might also be mediated by attenuation of the affective-motivational component of pain (Kauppila et al. 1991). Intra-articular dexmedetomidine also provides analgesia via α_{2a} -adrenoceptors (Al-Metwalli et al. 2008), enhancing the local anaesthetics' effects (Yoshitomi et al. 2008).

The sedative and analgesic effects of dexmedetomidine have been studied in depth in rats (Bol et al. 1999; Xu et al. 2000; Franken et al. 2008), cats (Ansah et al. 1998, 2000; Selmi et al. 2003; Granholm et al. 2006; Slingsby & Taylor 2008; Slingsby et al. 2009), dogs (Kuusela et al. 2000, 2001; Granholm et al. 2007; van Oostrom 2011; Lervik et al. 2012) and pigs (Sano et al. 2010). Its sedative effects were elicited at lower doses than those required for analgesia (Bol et al. 1999; Slingsby & Taylor 2008; van Oostrom 2011). Nevertheless, analgesia cannot be produced without sedation and sedation is not necessarily linked to comparative degrees of analgesia (Franken et al. 2008).

In dogs, the level of sedation did not differ between two different intravenous (IV) doses, (10-20 µg/kg) suggesting a ceiling effect, but the larger dose did have a longer duration of action (Kuusela et al. 2000). The sedative effects observed in cats were reported to be dose dependent after intramuscular (IM) administration (Ansah et al. 1998). However, when given IV, the dose dependent sedative effects were limited, and increases in serum concentrations beyond certain levels induced a reversal of sedation (Ansah et al. 2000). In pigs, dexmedetomidine enhanced the sedative effects of propofol (Sano et al. 2010).

Human reports conclude that the obtained degree of analgesia is not clearly dose dependent (Jaakola et al. 1991). The depth of sedation increases with higher doses with no clear advantages for analgesia (Hall et al. 2000). In dogs, a ceiling effect for both sedative and analgesic effects was seen when the drug was infused at two different rates (3-5 µg/kg/hr, IV) (van Oostrom et al. 2011). In cats, the degree of analgesia increased with higher drug concentrations, although the differences between analgesic scores at higher dose levels were narrow (Ansah et al. 2000). The analgesic effects of dexmedetomidine (20 µg/kg, IV) were longer than those of medetomidine in dogs (Kuusela et al. 2000), most likely because the levo-enantiomer competes with/antagonizes the dextro-form, or even acts on α_1 -adrenoceptors, reducing the sedative and analgesic effects (Kuusela et al. 2001). This suggests greater analgesic potency and predictability of dexmedetomidine, in agreement with the study of Granholm et al. (2007), where the analgesic scores were higher after IM dexmedetomidine, although the differences were not clinically appreciable. The use of low IV infusion rates was suggested to reduce the risk of post-operative chronic pain

development (Lervik et al. 2012). In cats, IM dexmedetomidine produced dose dependent analgesia (Ansah et al. 1998) comparable to that of medetomidine (Granholm et al. 2006). However, sedation may have influenced the reactions to stimuli (Ansah et al. 1998). Intramuscular dexmedetomidine at 40 µg/kg produced analgesia, whereas lower doses only produced dose dependent sedation (Slingsby & Taylor 2008). The oral transmucosal route may produce similar effects (Slingsby et al. 2009).

In order to provide good sedation and analgesia lasting beyond the procedure itself, lower doses are frequently used in combination with opioids (Selmi et al. 2003; Slingsby et al. 2010), mainly due to additive or synergistic effects (Ossipov et al. 1989).

2.4.2. Anaesthetic sparing effects

Sedation and analgesia probably account for the sparing effects on the minimum alveolar concentration (MAC) of volatile anaesthetic agents (Hall et al. 2000). In humans, IV dexmedetomidine decreased the MAC of isoflurane dose dependently (Aho et al. 1991; Aantaa et al. 1997). In dogs, IV boluses (1, 3 and 10 µg/kg) reduced clearly the MAC of halothane by 30, 60 and more than 90% respectively (Vickery et al. 1988). Furthermore, a 20 µg/kg IV bolus reduced the MAC of isoflurane in dogs by 86% (Weitz et al. 1991) and 89% (Bloor et al. 1992). When infused as a CRI, dexmedetomidine was shown to be a reliable and valuable adjunct to isoflurane (Uilenreef et al. 2008), while a CRI of 0.5 and 3 µg/kg/hr dexmedetomidine reduced isoflurane's MAC by 18 and 59% respectively in the canine species (Pascoe et al. 2006). Oral dexmedetomidine (15 µg/kg) reduced the MAC of halothane by 27% in cats (Schmeling et al. 1999) and, when given IV, the MAC of isoflurane decreased in a plasma concentration dependent manner (Escobar et al. 2012). The MAC of inhalants was also reduced when dexmedetomidine was given intraperitoneally in rats (Segal et al. 1988; Savola et al. 1991) or as an IV CRI (Rioja et al. 2006) and as an IV bolus in small ruminants (Kästner et al. 2007a).

Dexmedetomidine also reduced the requirements of injectable agents in humans (Venn et al. 1999; Scheinin et al. 1992) and animals (Salmenperä et al. 1994; Mendes et al. 2003). In human medicine, it improved patient comfort and postoperative analgesia when administered concurrently with opioids, resulting in a decreased need for additional pain medication (Arain et al. 2004; Unlugenc et al. 2005).

Also, when administered epidurally, dexmedetomidine produces MAC reductions of isoflurane in dogs (Campagnol et al. 2007) and cats (combined with lidocaine) (Souza et al. 2010). This route of administration increases the intensity and duration of analgesia, with reduced haemodynamic adverse effects (Campagnol et al. 2007).

2.4.3. Cardiorespiratory effects

The cardiovascular effects of dexmedetomidine result from both central and peripheral α_2 -adrenoceptor activity. In humans, dose dependent decreases in heart rate (HR) and \dot{Q}_t and a biphasic effect on mean arterial pressure, pulmonary arterial pressure and vascular resistance have been reported. Such a biphasic response is most clearly seen with high dose boluses and consists of bradycardia and hypertension due to initial stimulation of peripheral α_{2b} -vascular receptors, followed by central sympatholysis and a decline in blood pressure (Ebert et al. 2000). This has been considered to improve the haemodynamics of tachycardic, hypertensive patients. In contrast, these effects may be unwanted in compromised patients, whose \dot{Q}_t is rate dependent or with conduction system disease (Panzer et al. 2009).

In dogs, marked vasoconstriction and hypertension can be seen initially, with an increase in systemic vascular resistance and central venous pressure, followed by baroreflex-mediated bradycardia (Bloor et al. 1992) and a decrease in the cardiac index and oxygen delivery (Flacke et al. 1993). The following central sympatholysis and increase in parasympathetic tone leads to a reduction in systemic blood pressure, sustained bradycardia and decreases in cardiac index (Xu et al. 1998). These results can be explained by activation of central α_2 -adrenoreceptors, causing a reduction in sympathetic drive, with a presynaptically induced decrease of norepinephrine release at the sympathetic neuron terminals and activation of postsynaptic α_2 -receptors on vascular smooth muscle cells (Roekaerts et al. 1997). Dexmedetomidine does not seem to have a direct myocardial depressant effect (Flacke et al. 1992), although indirect suppression by catecholamine reduction has been suggested (Weitz et al. 1991; Flacke et al. 1993; Roekaerts et al. 1997). Cardiovascular changes in denervated dogs were reversed by atipamezole (Flacke et al. 1990). In cats, dose dependent decreases in HR and \dot{Q}_t , increases in total vascular resistance and transient mild changes in blood

pressure, are expected side effects of dexmedetomidine (Ansah et al. 1998, 2000; Selmi et al. 2003; Granholm et al. 2006).

In human medicine, dexmedetomidine produces limited respiratory effects, leading to a wide safety margin (Ebert et al. 2000; Venn et al. 2000). Due to its beneficial properties, dexmedetomidine was approved in the USA for procedural sedation in non-intubated patients in 2008 (Panzer et al. 2009). Decreases in respiratory rate (RR) with mild reductions in arterial oxygen tensions were reported after IV administration in dogs (Kuusela et al. 2000, 2001; Granholm et al. 2007). However, periods of short apnoea with slight cyanosis have been reported in dogs (Kuusela et al. 2000, 2001; Granholm et al. 2007). In cats, transient (Ansah et al. 1998; Granholm et al. 2006) or even non-significant (Selmi et al. 2003) decreases in RR were seen after IM administration. On the other hand, RR was not significantly affected by the dose in cats receiving different CRIs (Ansah et al. 2000). In small ruminants, dexmedetomidine (2 mg/kg, IV) produced decreases in arterial oxygenation, with goats being more sensitive than sheep due to the centrally mediated cardiovascular effects (Kutter et al. 2006). This was prevented by the use of a CRI in goats, but not in sheep, most likely due to individuals with high sensitivity (Kästner et al. 2007b).

2.4.4. Organ protective effects

Recently, there is increasing evidence that dexmedetomidine has organ protective effects against ischaemic and hypoxic injuries (Panzer et al. 2009). In human medicine, dexmedetomidine's neuroprotective properties, mediated by the α_{2a} -subtype, made its use popular in neuroanaesthesia (Ma et al. 2004; Bekker & Sturaitis 2005). Moreover, it produces vasoconstriction in cerebral vessels, decreasing cerebral blood flow (Zornow et al. 1993; Bekker & Sturaitis 2005) with no detrimental effects on local brain tissue oxygenation (Drummond & Sturaitis 2010). It also reduces the cerebrovascular dilation induced by isoflurane or sevoflurane in experimental dogs (Ohata et al. 1999) while intracranial pressure did not change in anaesthetized rabbits (Zornow et al. 1992) and dogs (Keegan et al. 1995) after administration of dexmedetomidine and medetomidine respectively. These properties make this drug a useful adjunct in inhalant anaesthesia in situations where increases in cerebral blood flow should be avoided (i.e. traumatic brain injury, large brain tumors).

In humans, α_2 -agonists decrease HR and arterial blood pressure during tachycardia and hypertension, suggesting their potential role in cardioprotection (Panzer et al. 2009). Dexmedetomidine reduced the mortality rate and the incidence of myocardial infarction after vascular surgery by reducing the degree of ischaemia (Wijeysundera et al. 2003), produced haemodynamic benefits (Talke et al. 1995) and decreased the incidence of ventricular arrhythmias compared to propofol (Herr et al. 2003). Moreover, its use was associated with a trend towards improved cardiac outcomes in non-cardiac surgery (Biccard et al. 2008) and with better outcomes in cardiac surgery (Ji et al. 2013). In experimental anaesthetized dogs, dexmedetomidine showed beneficial effects on ischaemic myocardium, preserving coronary blood flow, reducing oxygen demand and deficiency (Roekaerts et al. 1996a, b), while minimizing emergence-related myocardial ischaemia (Willigers et al. 2003). Moreover, it prevented halothane/epinephrine induced dysrhythmias in dogs (Hayashi et al. 1991), via the imidazoline receptors (Kamibayashi et al. 1995), while medetomidine failed to produce a similar response (Pettifer et al. 1996). After dexmedetomidine IV, experimental goats showed a better balance between myocardial oxygen supply and demand than other species (Lawrence et al. 1997). However, rising IV doses from 1 to 10 $\mu\text{g}/\text{kg}$ increased myocardial oxygen extraction, mediated by coronary vasoconstriction (Lawrence et al. 1996b). Moreover, first and second degree atrioventricular blocks were reported after IV dexmedetomidine in dogs (Kuusela 2000, 2001; Lervik et al. 2012) although ventricular arrhythmias were not detected more frequently (Kuusela et al. 2002).

With respect to other tissues, dexmedetomidine preserved flow to the most vital organs (brain, liver, kidneys) at the expense of less vital organs (Lawrence et al. 1996a). This is in agreement with reports on medetomidine which, when given IM, reduced intestinal and skeletal muscle blood flows, whereas renal cortical microvascular blood flow remained unaffected in isoflurane anaesthetized dogs (Pypendop & Verstegen 2000). In dogs, IM medetomidine increased the central nervous system (CNS) uptake of a lipophilic tracer during brain perfusion imaging (Waelbers et al. 2011). The authors postulated that the central distribution of the tracer might have been enhanced due to a lower proportionate decrease in CNS perfusion compared to peripheral tissues.

2.4.5. Other effects

Dexmedetomidine also induces adverse effects in animal species, such as vomiting in dogs and cats (Kuusela et al. 2000, 2001; Granholm et al. 2006; Slingsby & Taylor 2008) and uncontrolled elimination of urine and faeces in cats (Slingsby & Taylor 2008). Myoclonic twitching was seen in cats after administration of dexmedetomidine in combination with butorphanol, but not when given alone (Selmi et al. 2003). Furthermore, dexmedetomidine inhibited the transit in the small intestine in rats and guinea pigs (Asai et al. 1997; Herbert et al. 2002), increasing the gastrointestinal transit time.

Decreases in body temperature have been reported in dogs and cats (Ansah et al. 1998; Selmi 2003; Granholm et al. 2006, 2007) and may be of importance during long lasting anaesthetic procedures. These decreases are expected consequences of decreased heat production and muscular activity during sedation and direct effects on thermoregulation (Paris & Tonner 2005).

Diuresis is another relevant effect caused by inhibition of the antidiuretic hormone (Saleh et al. 2005; Villela et al. 2005). Moreover, medetomidine and dexmedetomidine altered blood glucose homeostasis via insulin inhibition by α_{2a} -adrenoceptors (Burton et al. 1997; Fagerholm et al. 2004) and modulated physiological stress responses (Benson et al. 2000; Kuusela et al. 2003). In humans, dexmedetomidine is also used to treat shivering in patients after regional and general anaesthesia (Talke et al. 1997; Bajwa et al. 2012).

2.5. Dexmedetomidine in horses

At the beginning of the present PhD, only the cardiopulmonary effects and pharmacokinetics of dexmedetomidine had been reported in ponies (Bettschart-Wolfensberger et al. 2005). A bolus of dexmedetomidine (3.5 $\mu\text{g}/\text{kg}$, IV) induced similar cardiopulmonary changes as the other α_2 -agonists but of very short duration. Heart rate and central venous pressure did not differ from baseline values for sixty minutes and the stroke volume was significantly reduced five minutes and cardiac index five to ten minutes after bolus administration. Arterial blood pressures were significantly increased for five minutes, reduced after twenty, thirty and forty five minutes and became normal at sixty minutes. Mean pulmonary arterial blood pressure

and systemic vascular resistance index were also increased five minutes after administration. Respiratory rate was reduced significantly but arterial blood gas tensions were not different compared to pre-sedation values. Dexmedetomidine plasma concentrations declined rapidly, falling beyond levels of sedation within sixty to ninety minutes. No more information was available about the use of dexmedetomidine in the equine at that time.

The idea of using dexmedetomidine as a CRI in horses seemed attractive as it has been described as a short-acting sedative with rapid redistribution (Bettschart-Wolfensberger et al. 2005). The use of medetomidine had been widely reported in horses with positive results (see Section 1 of the general introduction) while reports in dogs suggested benefits of dexmedetomidine over its racemate. In theory, the use of dexmedetomidine in equine balanced anaesthesia may provide sedation and analgesia, reducing the MAC of volatile agents and their potential side effects while improving the quality of recovery from general anaesthesia. However, potential side effects such as typical cardiopulmonary effects of α_2 -agonists, reduction of intestinal motility or increased diuresis inducing an overfilled bladder during general anaesthesia should be taken into consideration.

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Scientific aims

In order to reduce the dose dependent cardiovascular depression induced by volatile anaesthetics, combinations of different drugs can be used in equine anaesthetized patients. The objective of such combinations is to maintain a good intraoperative cardiopulmonary function followed by calm, smooth and coordinated recoveries. Dexmedetomidine is the most potent and selective α_2 -agonist marketed for small animals, and has been used intraoperatively in these species to provide sedation and analgesia and to reduce the amount of inhalant agents. At the beginning of the present PhD, only one study described the pharmacokinetics and cardiopulmonary effects after an intravenous bolus in ponies, favouring its use as constant rate infusion (CRI).

The overall aim of the present PhD was to investigate the inclusion of a CRI of dexmedetomidine in anaesthetized horses.

The **first objective** was to study the safety of two rates of dexmedetomidine CRIs in experimental ponies by detailed evaluation of the cardiopulmonary parameters. Using the results of this study in healthy ponies, the higher rate CRI protocol was studied in isoflurane anaesthetized equine patients, with the focus on the cardiopulmonary function and recovery quality.

The **second objective** was to assess to what extent the expired sevoflurane fraction could be reduced in the presence of a dexmedetomidine CRI. Additionally, the sevoflurane requirements were investigated when combined with either a morphine CRI or a combination of dexmedetomidine/morphine CRI.

The **third objective** was to compare the effects of a dexmedetomidine CRI with those of a morphine infusion in a clinical study, in order to determine potential benefits or disadvantages.

Experimental & clinical studies

Chapter 1
Cardiopulmonary effects of two constant rate
infusions of dexmedetomidine in isoflurane
anaesthetized ponies

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Cardiopulmonary effects of two constant rate infusions of dexmedetomidine in
isoflurane anaesthetized ponies. Vet Anaesth Analg 37, 311-321.

Abstract

Objective To evaluate the cardiopulmonary effects of two different constant rate infusions (CRI) of dexmedetomidine (1 and 1.75 $\mu\text{g}/\text{kg}/\text{hr}$) in experimental ponies.

Study design Prospective, randomized, experimental study.

Animals Six healthy ponies (mean $306 \pm \text{SD } 71$ kg, 7.0 ± 1.6 years).

Methods After premedication with intravenous (IV) dexmedetomidine (3.5 $\mu\text{g}/\text{kg}$), anaesthesia was induced (T0) with ketamine (2.2 mg/kg, IV) and midazolam (0.06 mg/kg, IV) and maintained with isoflurane (expiratory fraction of isoflurane 1.50 %) in 55 % oxygen for 150 minutes. Normocapnia was maintained using artificial ventilation. Three ponies received dexmedetomidine CRIs of 1 and 1.75 $\mu\text{g}/\text{kg}/\text{hr}$ from T30 to T60 and T90 to T120 respectively. In the other three ponies, the order of the doses was reversed. Continuous monitoring included pulse oximetry, electrocardiography, anaesthetic gas monitoring, arterial and central venous pressures. Cardiac output (LiDCO technique) was measured and arterial and venous bloods taken every fifteen minutes. Cardiac index (CI), systemic vascular resistance (SVR), arterial and venous oxygen content (CaO_2 , CvO_2) and oxygen delivery (DO_2) were calculated. Analysis of variance with separate models for each CRI rate was used to detect differences between values obtained at the end of the CRI and their respective baseline values. A mixed model with these differences as response variable, pony as random effect and treatment and period as fixed effects was applied to find differences between the two CRIs ($\alpha = 0.05$ for all analyses).

Results Heart rate (HR), CI, CaO_2 , CvO_2 and DO_2 decreased significantly, while significant increases were found in SVR, systolic arterial pressure and right atrial pressure with both infusion rates. No differences were found between the two dexmedetomidine CRI rates.

Conclusions and clinical relevance Although significant, cardiopulmonary effects of the dexmedetomidine CRIs in isoflurane anaesthetized ponies were minimal, without differences between the two dose rates.

Introduction

General anaesthesia carries a higher risk of mortality in horses compared to small animals and humans (Johnston et al. 2002). Inhalation anaesthesia is used commonly for long procedures. The incidence of deaths resulting directly or indirectly from anaesthesia may be related at least partly to the dose dependent cardiovascular depression induced by inhalation anaesthetics (Steffey & Howland 1980). Constant rate infusions (CRIs) of different drugs can provide analgesia and increase anaesthetic depth during surgical interventions, reducing the requirement for inhalant anaesthetics. Lidocaine (Doherty & Frazier 1998), ketamine (Muir & Sams 1992) and different α_2 -agonists (Wagner et al. 1992; Kuhn et al. 2004; Ringer et al. 2007) have been used as CRIs in anaesthetized horses for this purpose. Alpha₂-agonists are potent sedatives with good analgesic properties and reduce the minimum alveolar concentration (MAC) of inhalation anaesthetic agents (England & Clarke 1996; Steffey et al. 2000; Bettschart-Wolfensberger et al. 2001, 2005). Classic side effects of α_2 -agonists in horses, include bradycardia, arrhythmias, a decrease in cardiac output and an increase in vascular resistance (England & Clarke 1996; Yamashita et al. 2000), but despite these effects, the agents have been accepted for use in balanced anaesthetic protocols. The most prominent side effects of the most commonly used α_2 -agonists occur following an intravenous (IV) bolus. However, under steady state conditions of medetomidine infusions in ponies, cardiac index (CI) and systemic vascular resistance index (SVRI) were reported not to be different from presedation values (Bettschart-Wolfensberger et al. 1999a).

Xylazine, detomidine and romifidine have a marketing authorisation for use in horses in countries of the European Union. Medetomidine is a potent and selective α_2 -agonist (Pertovaara 1993; Bryant & Clarke 1996; Virtanen et al. 1998) but is licensed specifically for small animals. However, its use as a CRI during inhalation anaesthesia in horses has been investigated, not only under experimental conditions (Bettschart-Wolfensberger et al. 2001), but also in clinical circumstances (Kalchofner et al. 2006). A medetomidine CRI provides good intra-operative analgesia, stable cardiopulmonary function and a rapid, good quality recovery (Bettschart-Wolfensberger et al. 1999a, b) and its pharmacokinetics make it suitable for prolonged use by infusion (Bettschart-Wolfensberger et al. 1999b).

Dexmedetomidine, the dextro-rotary, active enantiomer of medetomidine, provides more analgesia compared to an equivalent dose of the racemic mixture in dogs (Kuusela et al. 2000). The plasma half-life of dexmedetomidine is shorter than that of medetomidine, not only in dogs (Kuusela et al. 2000) but also in ponies (Bettschart-Wolfensberger et al. 2005). In ponies, dexmedetomidine was more rapidly redistributed compared to humans, the cause being attributed to a larger volume of distribution (Bettschart-Wolfensberger et al. 2005). Additionally, studies in humans and dogs demonstrated that dexmedetomidine results in a significant reduction of the MAC of isoflurane (Aantaa et al. 1997; Pascoe et al. 2006). To the authors' knowledge, the effects of a dexmedetomidine CRI under clinical circumstances have only been studied in dogs (Uilenreef et al. 2008) and small ruminants (Kästner et al. 2007).

The cardiopulmonary effects and pharmacokinetics of an IV bolus of dexmedetomidine have been reported in experimental ponies, whereby a dose of 3.5 µg/kg dexmedetomidine was considered to be equivalent to 7 µg/kg medetomidine. The observed cardiopulmonary effects were similar to those induced by other α_2 -agonists, but of shorter duration (Bettschart-Wolfensberger et al. 2005).

The effects of dexmedetomidine as a CRI have not been documented in horses or ponies in conjunction with inhalation anaesthesia. The objective of the present study was to determine the cardiopulmonary effects of a thirty minute infusion of dexmedetomidine, administered at two different dose rates, in isoflurane anaesthetized ponies.

Materials and methods

The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine of the University of Ghent (2008/005).

Animals and instrumentation

Six healthy ponies (five geldings and one mare), aged 7.0 ± 1.6 years and weighing 306 ± 71 kg, were included in this study. The left carotid artery had been transposed to a subcutaneous position at least two years before the experiments. Food, but not water, was withheld for twelve hours before general anaesthesia.

Following subcutaneous administration of a local anaesthetic (2 mL mepivacaine, Scandicaine 2%, Astrazeneca, Belgium), a 5 Fr Swan-Ganz catheter was inserted into the right jugular vein, with the distal port of the catheter in the right atrium. Correct positioning of the catheter was confirmed using the characteristic pressure waveforms, visualised on a cardiovascular monitor. A venous blood sample was taken for determination of plasma sodium concentration.

The ponies were then sedated with dexmedetomidine (3.5 µg/kg, IV). A 14-gauge catheter was placed in the left jugular vein. Fifteen minutes later, anaesthesia was induced with IV midazolam 0.06 mg/kg (Dormicum, Roche, Belgium) and ketamine 2.2 mg/kg (Anesketin, Eurovet, Belgium) mixed in the same syringe. After endotracheal intubation (24 or 26 mm tube), the ponies were positioned in right lateral recumbency on a surgical table and were supported by soft foam rubber pillows (twenty cms). The endotracheal tube was connected to a large animal anaesthetic unit (Matrx Medical Inc., NY, USA mounted on a Sulla 909V, Dräger, Germany) with an out-of-circuit vaporizer (Drägerwerk AG) and a large animal ventilator (Smith respirator LA 2100, model 2002, Veterinary Technics/BDO-Medipass, The Netherlands). Anaesthesia was maintained with isoflurane (Isoflo, Abbott Laboratories Ltd., UK) in a mixture of oxygen (O₂) and air [inspired oxygen fraction (FiO₂) 55 %]. Isoflurane vaporizer setting was adjusted to maintain an expiratory fraction of isoflurane (F_E ISO) of 1.50%. Respiration mode was 'assisted-controlled', with a tidal volume of 10 mL/kg, respiratory rate (RR) of 10 breaths/min, peak inspiratory pressure of 1.96 kPa (20 cmH₂O) and inspiration time of 1.8 seconds. The settings were then adjusted to maintain arterial partial pressure of carbon dioxide (PaCO₂) between 4.66 and 6.00 kPa (35 and 45 mmHg).

Lactated Ringer's solution (Ringer Lactate, Vetoflex, France) was infused during the anaesthetic period at a rate of 5 mL/kg/hr using two volumetric pumps (Colleague, Baxter Healthcare Corporation, IL, USA). A urinary catheter was placed in all ponies.

The skin over the transposed left carotid artery was surgically prepared and a 22-gauge catheter (Vasocan Braunüle Luer Lock, B. Braun Melsungen AG, Germany) was placed in the artery and connected to a pressure transducer (placed at the level of the right atrium). The distal port of the Swan Ganz catheter was connected to a second

pressure transducer. The pressure monitoring system was calibrated against a mercury manometer before each experiment and zeroed at the level of the right atrium.

Inspiratory and expiratory CO₂, O₂ and isoflurane concentrations were measured from gas withdrawn continuously from the Y-piece of the anaesthetic circuit, using a calibrated, methane-insensitive, multi-gas analyzer (HP M1025B, Hewlett Packard Company, TX, USA). A CMS-Patient monitor (Hewlett-Packard, GmBh, Germany) was used to record the ECG (base-apex lead), to monitor systolic (SAP), diastolic (DAP), mean arterial (MAP) and right atrial pressure (RAP), to perform pulse oximetry (probe placed on the tongue) and to measure body temperature using an oesophageal probe. Cardiac output was obtained with the lithium dilution technique (LiDCOplus Haemodynamic Monitor, LiDCO Ltd.). A one mL bolus of lithium chloride (1.5 mmol/mL) was injected in the central venous circulation through the proximal port of the thermodilution catheter for each measurement. Haemoglobin concentration was calculated from the packed cell volume (PCV) measured fifteen minutes after induction (T15) using the following formula (Linton et al. 2000): $Hb (g/dL) = 34 \times PCV (L/L)$.

Experimental design

The ponies were divided randomly into two treatment groups. Each pony was anaesthetized once only, for a period of 150 minutes. T0 was the time when the ponies were connected to the anaesthetic circuit. Ponies from treatment 1 (ponies 1, 3 and 5) received a CRI of 1 µg/kg/hr dexmedetomidine between T30 and T60 (period A) and 1.75 µg/kg/hr between T90 and T120 (period B). In treatment 2 (ponies 2, 4, 6), the order of the doses was reversed. A syringe driver was used to administer the dexmedetomidine CRIs. Values recorded at T30 and T90 were regarded as baseline values for periods A and B respectively.

Values for inspiratory and expiratory CO₂ and O₂, heart rate (HR), SAP, MAP, DAP, RAP and body temperature were recorded at five minute intervals throughout anaesthesia (until T150). Cardiac output was measured and arterial and central venous blood samples were collected for immediate analysis at fifteen minute intervals (ABL5, Radiometer, Denmark). Blood gas data were corrected for body temperature.

Cardiac index, stroke volume (SV), stroke volume index (SVI), arterial oxygen content (CaO₂), central venous oxygen content (CvO₂), degree of venous admixture,

oxygen delivery (DO_2) and oxygen consumption were calculated using standard formulas (Schauvliege et al. 2008).

After T150, all catheters were removed and the ponies were placed in right lateral recumbency in a padded recovery box and allowed to recover without assistance or additional sedation. Oxygen was insufflated (flow rate of 8 L/min) through the endotracheal tube and nasally after extubation. The endotracheal tube was removed once the ponies were able to swallow. Extubation time, time to sternal recumbency and time to stand were recorded. A score, on a scale of 1-5 (Table 1) was awarded for the quality of recovery.

Table 1: Scoring system used to grade recovery of experimental ponies.

Score	Description
1	One attempt to stand, no ataxia.
2	One to two attempts to stand, some ataxia.
3	More than two attempts to stand but quiet recovery.
4	More than two attempts to stand, excitation.
5	Severe excitation. Pony injured.

Statistical analysis

An analysis of variance with separate models for each CRI rate was used to detect differences between values obtained at the end of the CRI and their respective baseline values. These differences were used as the response variable in an analysis of variance with period as fixed effect, and separate models were fitted for the 1 and 1.75 $\mu\text{g}/\text{kg}/\text{hr}$ CRIs. The significance level was set at 5%. Additionally, a mixed model with the difference as response variable, pony as random effect and treatment and period as fixed effects was applied to evaluate the difference between the two treatments. A paired t-test was performed to compare the two baseline periods (T30 versus T90).

Results

Cardiovascular system (Table 2)

The paired t-tests performed between the two baselines demonstrated that all the parameters were comparable except HR (decreasing 3 ± 2 beats/min from T30 to T90) and RAP (increasing 4 ± 3 mmHg from T30 to T90).

At the end of the $1 \mu\text{g/kg/hr}$ CRI, HR and CI decreased significantly [mean decreases of 3 ± 1 beats/min ($p = 0.02$) and 4.9 ± 0.3 mL/kg/min ($p = 0.0001$) respectively], while SAP increased significantly [mean increase of 8 ± 3 mmHg ($p = 0.047$)] compared to the respective baseline values. Similar decreases in HR and CI were found following the $1.75 \mu\text{g/kg/hr}$ CRI [mean decreases of 3 ± 0 beats/min ($p = 0.0001$) and 7.7 ± 2.4 mL/kg/min ($p = 0.02$) respectively], while RAP increased significantly [mean increase of 2 ± 1 mmHg ($p < 0.0446$)]. No significant differences for any of the measured variables were found between the two different CRI rates.

At T60, after period A (T30 to T60), HR and CI decreased significantly [mean decrease of 4 ± 0 beats/min ($p = 0.001$) and 6.7 ± 1.8 mL/kg/min ($p = 0.02$) respectively] compared with the respective baseline values (T30). At T120, after period B (T90 to T120), HR and CI were significantly lower [mean decrease of 2 ± 0 beats/min ($p = 0.01$) and 5.9 ± 1.8 mL/kg/min ($p = 0.03$) respectively] while SAP, MAP and DAP were significantly higher [mean increases of 11 ± 2 mmHg ($p = 0.01$), 10 ± 3 mmHg ($p = 0.02$) and 9 ± 3 mmHg ($p = 0.03$) respectively]. Significant differences between periods A and B were found for HR ($p = 0.02$), SAP ($p = 0.05$) and RAP ($p = 0.03$).

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Table 2: Cardiovascular parameters in six isoflurane anaesthetized ponies supplemented with constant rate infusions of dexmedetomidine.

Values	Differences	Unit	Group	Period A		Period B		
				T30	T60	T90	T120	T150
HR	*, §, #	beats/min	1	43 ± 3	39 ± 3	39 ± 1	37 ± 1	39 ± 3
			2	42 ± 1	39 ± 1	39 ± 1	38 ± 1	38 ± 0
SAP	*	mmHg	1	80 ± 5	84 ± 5	80 ± 9	91 ± 10	82 ± 13
			2	76 ± 5	75 ± 9	71 ± 9	82 ± 6	83 ± 10
MAP		mmHg	1	63 ± 6	67 ± 5	64 ± 10	76 ± 14	66 ± 13
			2	56 ± 3	57 ± 6	57 ± 6	65 ± 6	67 ± 11
DAP		mmHg	1	52 ± 5	55 ± 4	54 ± 10	65 ± 15	56 ± 14
			2	47 ± 3	48 ± 6	49 ± 6	55 ± 6	58 ± 12
RAP	§, #	mmHg	1	13 ± 2	17 ± 3	17 ± 2	18 ± 4	17 ± 3
			2	18 ± 5	20 ± 4	22 ± 7	21 ± 6	21 ± 7
SVR		dyne/sec/cm ⁵	1	242 ± 40	268 ± 26	250 ± 73	393 ± 190	286 ± 100
			2	181 ± 53	201 ± 73	173 ± 29	244 ± 63	256 ± 94
CI	*, §	mL/kg/min	1	59.8 ± 7.4	54.5 ± 7.8	56.5 ± 13.6	49.1 ± 19.6	51.8 ± 12.5
			2	56.2 ± 15.7	48.2 ± 10.3	51.7 ± 14.0	47.2 ± 13.4	47.3 ± 13.3
SI		mL/kg	1	1.4 ± 0.3	1.4 ± 0.3	1.4 ± 0.4	1.3 ± 0.5	1.3 ± 0.4
			2	1.3 ± 0.4	1.2 ± 0.2	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3

Heart rate (HR), systolic (SAP), diastolic (DAP), mean arterial (MAP) and right atrial (RAP) pressures, systemic vascular resistance (SVR), cardiac index (CI) and stroke index (SI) in six isoflurane anaesthetized ponies supplemented with constant rate infusions (CRIs) of dexmedetomidine. Ponies of Group 1 (n = 3) received dexmedetomidine 1 µg/kg/hr from T30 until T60 (period A) and 1.75 µg/kg/hr from T90 until T120 (period B). In ponies of Group 2 (n = 3) the order of the infusion rates was reversed. Data are represented as mean ± SD.

* Value after dexmedetomidine 1 µg/kg/hr significantly different from respective baseline values (p < 0.05). § Value after dexmedetomidine 1.75 µg/kg/hr significantly different from respective baseline values (p < 0.05). # Significant differences were found between baseline periods (T30 and T90).

Body temperature (Table 3)

Body temperature decreased significantly between the two baseline measurements ($0.3 \pm 0.1^{\circ}\text{C}$ from T30 to T90). Body temperature decreased significantly during all treatments and periods. No differences between treatments or periods were found.

Blood analyses (Table 3)

There were some statistically significant differences between the baseline periods in parameters from blood analyses. Arterial oxygen tension (PaO_2) and PCV both decreased [4.3 ± 2.8 kPa (33 ± 21 mmHg); $2 \pm 2\%$ respectively] from T30 to T90.

Packed cell volume decreased significantly [mean decrease of $2 \pm 0\%$ ($p = 0.02$)] at the end of the $1 \mu\text{g}/\text{kg}/\text{hr}$ CRI compared with the respective baseline values. The $1 \mu\text{g}/\text{kg}/\text{hr}$ CRI had little influence on the PaO_2 in both treatment groups [(16.1 ± 6.3 versus 16.5 ± 6.3 kPa (121 ± 47 versus 124 ± 47 mmHg) in treatment 1, 7.2 ± 2.9 versus 7.7 ± 3.7 kPa (54 ± 22 versus 58 ± 28 mmHg) in treatment 2]. In contrast, the $1.75 \mu\text{g}/\text{kg}/\text{hr}$ CRI induced significant decreases in arterial and venous PO_2 [overall mean decrease of 3 ± 1 kPa (26 ± 8 mmHg) ($p = 0.02$) and 1 ± 0 kPa (5 ± 2 mmHg) ($p = 0.03$) respectively]. No differences between the two CRI rates were found.

At T60, after period A (T30 to T60), PCV was significantly lower [mean decrease of $2 \pm 0\%$ ($p = 0.04$)]. Venous pH decreased significantly [mean decrease of 0.02 ± 0.00 ($p < 0.02$)] at T120, after period B (T90 to T120) compared with baseline values. No differences between the two periods were found.

Other calculated values (Table 3)

When comparing the two baseline periods using paired t-tests significant decreases in CaO_2 (18 ± 10 mL/L), CvO_2 (19 ± 9 mL/L) and DO_2 (0.46 ± 0.31 mL/kg/min) were found.

Arterial oxygen content, CvO_2 and DO_2 were significantly lower [mean decrease of 7 ± 2 mL/L ($p = 0.04$), 13 ± 4 mL/L ($p < 0.02$) and 0.97 ± 0.39 mL/kg/min ($p < 0.002$) respectively] at the end of the $1 \mu\text{g}/\text{kg}/\text{hr}$ CRI compared with the baseline values. After the $1.75 \mu\text{g}/\text{kg}/\text{hr}$ CRI, only the decreases in CvO_2 and DO_2 were significant [mean decrease of 12 ± 3 mL/L ($p = 0.01$) and 1.21 ± 0.46 mL/kg/min ($p = 0.01$) respectively]. No differences between the two doses were found.

At T60, after period A (T30 to T60), CaO_2 , CvO_2 and DO_2 were significantly lower [mean decrease of 12 ± 3 mL/L ($p = 0.03$), 14 ± 4 mL/L ($p = 0.02$) and 1.37 ± 0.32 mL/kg/min ($p = 0.01$) respectively] compared with the respective baseline values. Only CvO_2 and DO_2 were significantly decreased at T120, after period B (T90 to T120) [mean decreases of 11 ± 4 mL/L ($p = 0.04$) and 0.86 ± 0.39 mL/kg/min ($p < 0.01$) respectively]. No differences between periods were found.

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Table 3: Other parameters in six isoflurane anaesthetized ponies supplemented with constant rate infusions of dexmedetomidine.

Values	Differences	Unit	Group	Period A		Period B		
				T30	T60	T90	T120	T150
Body temperature	*, §, #	°C	1	36.2 ± 0.3	36.0 ± 0.2	35.8 ± 0.2	35.6 ± 0.2	35.5 ± 0.2
			2	36.1 ± 0.3	36.0 ± 0.3	35.9 ± 0.2	35.8 ± 0.2	35.6 ± 0.2
Venous pH			1	7.48 ± 0.06	7.47 ± 0.05	7.47 ± 0.04	7.46 ± 0.04	7.47 ± 0.05
			2	7.47 ± 0.06	7.46 ± 0.05	7.46 ± 0.03	7.44 ± 0.03	7.44 ± 0.02
Venous PCO ₂		kPa	1	5.6 ± 0.8	5.6 ± 1.2	5.7 ± 1.0	6.0 ± 1.2	5.6 ± 1.0
			2	6.0 ± 1.2	6.1 ± 0.9	6.1 ± 0.4	6.5 ± 0.8	6.7 ± 0.2
Venous PCO ₂		mmHg	1	42 ± 6	42 ± 9	43 ± 8	45 ± 9	42 ± 8
			2	45 ± 9	46 ± 7	46 ± 3	49 ± 6	50 ± 2
Venous PO ₂	§	kPa	1	6.1 ± 2.6	4.8 ± 1.0	5.0 ± 0.9	4.2 ± 0.5	4.5 ± 0.5
			2	4.3 ± 0.0	3.7 ± 0.2	3.4 ± 0.5	3.3 ± 1.1	3.5 ± 1.2
Venous PO ₂	§	mmHg	1	46 ± 20	36 ± 8	38 ± 7	32 ± 4	34 ± 4
			2	32 ± 0	28 ± 2	26 ± 4	25 ± 8	26 ± 9
Arterial pH			1	7.50 ± 0.05	7.50 ± 0.05	7.49 ± 0.04	7.49 ± 0.04	7.49 ± 0.05
			2	7.50 ± 0.09	7.50 ± 0.06	7.49 ± 0.03	7.48 ± 0.04	7.47 ± 0.03
Arterial PCO ₂		kPa	1	5.3 ± 0.8	5.3 ± 0.8	5.3 ± 0.7	5.6 ± 0.8	5.6 ± 0.8
			2	5.6 ± 1.3	5.3 ± 1.2	5.6 ± 0.8	5.9 ± 0.7	6.1 ± 0.3
Arterial PCO ₂		mmHg	1	40 ± 6	40 ± 6	40 ± 5	42 ± 6	42 ± 6
			2	42 ± 10	40 ± 9	42 ± 6	44 ± 5	46 ± 2
Arterial PO ₂	§, #	kPa	1	16.1 ± 6.3	16.5 ± 6.3	13.1 ± 4.3	10.5 ± 3.6	10.4 ± 6.1
			2	12.8 ± 5.9	8.5 ± 3.7	7.2 ± 2.9	7.7 ± 3.7	8.4 ± 4.1
Arterial PO ₂	§, #	mmHg	1	121 ± 47	124 ± 47	98 ± 32	79 ± 27	78 ± 46
			2	96 ± 44	64 ± 28	54 ± 22	58 ± 28	63 ± 31
Arterial oxygen content	*, #	mL/L	1	133 ± 17	124 ± 13	116 ± 14	113 ± 11	112 ± 21
			2	122 ± 23	108 ± 11	103 ± 13	98 ± 20	100 ± 17
Venous oxygen content	*, §, #	mL/L	1	109 ± 23	93 ± 22	92 ± 21	80 ± 19	61 ± 57
			2	85 ± 13	73 ± 18	63 ± 7	53 ± 18	54 ± 15
Venous admixture		%	1	42 ± 21	29 ± 9	38 ± 14	33 ± 13	39 ± 27
			2	30 ± 3	40 ± 20	40 ± 12	38 ± 5	35 ± 5
Oxygen delivery index	*, §, #	mL/kg/min	1	8.03 ± 1.92	6.83 ± 1.61	6.67 ± 2.24	5.68 ± 2.71	6.00 ± 2.42
			2	6.65 ± 0.87	5.10 ± 0.62	5.22 ± 1.22	4.49 ± 1.13	4.62 ± 0.97
Oxygen consumption index		mL/kg/min	1	1.51 ± 1.16	1.75 ± 0.80	1.44 ± 0.87	1.57 ± 0.72	1.41 ± 0.80
			2	1.98 ± 0.2	1.72 ± 0.67	1.98 ± 0.22	1.88 ± 0.46	1.98 ± 0.34
Packed cell volume	*, #	%	1	28 ± 4	26 ± 3	25 ± 4	24 ± 3	25 ± 4
			2	26 ± 6	25 ± 6	25 ± 7	24 ± 8	24 ± 7

* Value after dexmedetomidine 1 µg/kg/hr significantly different from respective baseline values (p < 0.05). § Value after dexmedetomidine 1.75 µg/kg/hr significantly different from respective baseline values (p < 0.05). # Significant differences were found between baseline periods (T30 and T90).

Recovery

Extubation times were 11.67 ± 4.16 and 11.00 ± 3.61 minutes for treatments 1 and 2 respectively. Time to standing ranged between fifteen and thirty minutes. For ponies of treatment 1 mean time to standing was 20.33 ± 3.06 minutes while the recovery time in group 2 was 17.00 ± 3.46 minutes. All ponies had good recoveries (scores 1 or 2) with minimal or no ataxia.

Discussion

The present study aimed at identifying cardiopulmonary effects of two different CRI rates of dexmedetomidine in isoflurane anesthetized ponies. Overall, the results of the present study demonstrated that a CRI of dexmedetomidine can be applied without major side effects in isoflurane anesthetized ponies. Moreover, both infusion rates induced similar cardiopulmonary effects. Although statistical differences were detected for some parameters such as HR and CI, these differences were small and probably of only limited importance in healthy animals.

The design of the present study, aimed at reducing the time period of the experiment and limiting the number of anaesthetic episodes per pony, imposed some limitations. The results obtained may have been influenced by the simultaneous administration of other anaesthetic drugs used for premedication and induction. However, the main objective of the present study was to evaluate the effects of a CRI of dexmedetomidine in experimental ponies under standardized conditions, using an anaesthetic protocol that can be routinely applied in clinical patients. Nevertheless, the effects of time, possible carry-over effects between the two periods and differences in body conformation between ponies may have had a major impact on the data.

Theoretically, a loading dose of dexmedetomidine should be included into the protocol of the CRI in order to quickly achieve adequate plasma concentrations. However, no loading dose was given, as it is probable that the administration of a bolus of dexmedetomidine before each infusion would have caused major cardiopulmonary changes which would have overshadowed the effects of the CRI and potential differences between the CRI rates. Furthermore, the cardiovascular effects of a bolus of an α_2 -agonist might be dangerous during a relatively deep level of isoflurane anaesthesia (F_E ISO of 1.50 %) used in the present study, which would also be hard to

justify during clinical anaesthesia in horses. In the clinical situation, the dose administered for premedication is usually regarded as the loading dose. In the present study, dexmedetomidine was administered as premedication fifteen minutes before induction of anaesthesia. Although this would have had an effect on the cardiopulmonary system, the effects of dexmedetomidine after IV injection were reported to be short-lasting (Bettschart-Wolfensberger et al. 2005). Consequently, the effects of the dexmedetomidine administered as premedication in the present study should have been minimal by T30, when the first CRI was initiated. Nevertheless, with a loading dose administered forty five minutes before initiating the CRI, steady state plasma concentrations should still have been reached earlier than if no loading dose had been given at all.

Cardiovascular function was only evaluated at the end of each infusion period of thirty minutes as during the initial stages of the infusion plasma concentrations of dexmedetomidine would have been low. Rough calculations using the available pharmacokinetic data in ponies (Bettschart-Wolfensberger et al. 2005) suggest that a steady state can only be reached after about eighty to hundred minutes. Although steady state plasma concentrations probably were not reached by the end of the infusion periods, most of the recorded values appeared to change mainly during the initial phase of the infusion and were quite stable towards the end of that time period, suggesting that most of the effects were maximal at that time.

Possible carry-over effects between infusion periods may have occurred in the present study design. To exclude this possibility T30 and T90 were compared using paired t-tests for the different parameters. Cardiovascular parameters were similar at these points, except for minor changes in HR and RAP. Changes in arterial blood gases (PaO₂ and PCV) and calculated values (CaO₂, CvO₂ and DO₂) were minimal and could be explained by the effect of time. Although the pharmacokinetics of dexmedetomidine in isoflurane anaesthetized ponies have not been investigated, its cardiovascular effects after a bolus injection in standing ponies were short lasting (Bettschart-Wolfensberger et al. 2005), suggesting that a washout period of thirty minutes after the end of a first CRI was acceptable. This assumption was confirmed in the present study by the comparable baseline values recorded in the two time periods.

Isoflurane affects cardiovascular function and these changes are influenced by the duration of anaesthesia. However, in a study in horses in which, following sedation with romifidine, anaesthesia was induced with ketamine and maintained with isoflurane, systemic vascular resistance (SVR) increased gradually over time while CI decreased progressively (Raisis et al. 2005). Ringer et al. (2007) investigated the effects of a CRI of medetomidine in horses undergoing anaesthesia for clinical purposes. Over time, SVR and arterial blood pressures increased as did CI, albeit it from an initial low level which possibly resulted from the effect of the bolus of medetomidine used for initial sedation. In the present study, these temporal effects may also have influenced the data obtained, although randomization of the order of treatments should have minimized this effect. In a clinical setting, $F_E \text{ ISO}$ would be lower than that used in the present study due to considerable isoflurane-sparing effects of dexmedetomidine. This might favour lower CRI rates with regards to haemodynamic performance, as a higher isoflurane requirement will somewhat obtund the increase in SVR, resulting in decreased cardiac work and myocardial oxygen consumption for the same level of CI.

The cardiovascular effects of ketamine used for induction of anaesthesia should also be considered, as these may again have influenced measured values, especially the ones recorded at T30 which were used as baseline. Although ketamine's direct effect on the heart is depressant (Graf et al. 1995), it has been shown to increase the sympathetic efferent activity, hereby increasing HR, arterial blood pressure (Wong & Jenkins 1974) and myocardial oxygen consumption (Bålfors et al. 1983). Nevertheless, these stimulating effects may have been counteracted in the present study by the dexmedetomidine sedation or even the administration of midazolam.

In equine anaesthesia, maintenance of cardiac performance is of major importance to guarantee a sufficient muscle blood flow and oxygenation, thereby reducing the potential risk of post-anaesthetic myopathy (Lee et al. 1998). Good oxygen delivery is also critical to prevent tissue hypoxia. After thirty minutes of anaesthesia, a marked decrease in PaO_2 was present in all ponies. It is suggested that this was due to ventilation/perfusion mismatch as the ponies used in the present study were round bellied and relatively fat and heavy body weight ponies are prone to these problems (Moens 1989).

The sedation dose used in these experimental ponies was based on studies where 7 µg/kg medetomidine was estimated to be equipotent to 3.5 µg/kg dexmedetomidine (Bettschart-Wolfensberger et al. 2005). All ponies were well sedated, with the typical lowering of the head, and the degree of ataxia was acceptable. Induction of anaesthesia with the standard drugs and subsequent endotracheal intubation were uneventful.

No effective calculations were performed to obtain the optimal dose needed for CRI of dexmedetomidine. Dexmedetomidine CRI dosages have not been reported for equines previously. Similar studies in standing horses with, after an initial bolus dose, a medetomidine infusion rate of 3.5 µg/kg/hr gave good sedation and resulted in stable blood levels of the drug with acceptable cardiopulmonary effects and reduced the MAC of desflurane (Bettschart-Wolfensberger et al. 1999a, b; 2001). Based on the above mentioned equipotent doses of medetomidine and dexmedetomidine, we chose two different infusion rates: 1.75 µg/kg/hr dexmedetomidine was estimated as being equivalent to 3.5 µg/kg/hr medetomidine, while a lower infusion rate of 1 µg/kg/hr was also studied. Future pharmacokinetic studies are justified to determine whether constant and effective plasma levels can be achieved with both doses.

In this present study no clear differences between the two CRI rates were found. This might be due to a 'ceiling' of both the positive and negative effects of the dexmedetomidine CRIs. Indeed, the analgesic and sedative effects have their upper limits, whereby increasing the dose only extends the duration of sedation and analgesia. Such an effect has been described also for the cardiovascular side effects of different doses of romifidine (Pypendop & Verstegen 2001). Consequently, cardiovascular effects may already occur at a low CRI dose, without further dose related increases.

Cardiovascular function was well maintained in both treatments of ponies receiving a CRI of dexmedetomidine. Overall, HR significantly decreased with both infusion rates, as a typical effect of the α_2 -agonists. Activation of α_{2b} -receptors has been reported to induce an initial surge in vascular resistance followed by a reflex bradycardia (Maze & Tranquili 1991; Maze & Fujinaga 2000; Guimarães & Moura 2001). More recently, it has been shown that α_2 -agonists additionally induce bradycardia by central mechanisms (Enouri et al. 2008; Honkavaara et al. 2008). However, the observed decrease in HR was minimal and no periods of severe bradycardia or atrio-ventricular blocks were observed in the ponies. The decrease in HR

was significantly more pronounced by the end of period A (from T30 to T60) compared to the end of period B (from T90 to T120) in both treatments but this difference was not of clinical relevance (2 beats/min).

Baseline MAP values were low in the present study compared to those considered acceptable during clinical anaesthesia. This was presumed to be due to the deep level of isoflurane anaesthesia (F_E ISO 1.50%) and resultant decrease in SVR (Rödig et al. 1996). No surgical stimulation was performed which may have counteracted this decrease. Decreased cardiac compensation could also explain the low arterial pressures observed in the present study. Indeed, inhibitory effects of dexmedetomidine on release of norepinephrine (Ebert et al. 2000) may potentiate depressive influences induced by the volatile agents (vasodilation and myocardial depression).

Arterial pressure increased after infusion of dexmedetomidine, the most probable cause being vasoconstriction induced by the activation of α_{2b} -adrenoceptors on endothelial smooth muscle, resulting in an increase in SVR (Maze & Tranquili 1991; Maze & Fujinaga 2000; Guimarães & Moura 2001). Mean arterial pressure and SVR increased significantly in period B compared with baseline values. Differences between both infusion rates and periods were not observed. However, the increases in MAP and SVR were only significant for period B and not for period A and so this is most likely explained by the accumulation of dexmedetomidine over the two infusion periods of thirty minutes and by time related changes. Compared to the respective baseline values, CI decreased in both treatment groups of ponies receiving dexmedetomidine CRIs, mainly attributable to a decrease in HR. Both 1 and 1.75 $\mu\text{g}/\text{kg}/\text{hr}$ CRIs induced a decrease in CI, which returned to baseline values during the 'washout' period when no dexmedetomidine was administered.

In the present study significant decreases in CI and DO_2 occurred after the dexmedetomidine CRIs, but these decreases were approximately 10% of baseline values. Such decreases are usually well tolerated in healthy anaesthetized horses under clinical conditions. Also under clinical circumstances, the concentrations of isoflurane would have been reduced once dexmedetomidine infusion commenced. Nevertheless, caution should be taken in compromised horses. Even more, the observed decrease in arterial and venous PO_2 throughout the anaesthesia in the present study might also have

an influence on the other parameters. The purpose of using an FiO_2 of 55% was to reduce the degree of atelectasis which might occur (Marntell et al. 2005) but in this case it was not successful in avoiding substantial pulmonary right to left shunting. In a clinical case the FiO_2 would have been increased.

In both treatments, core temperature decreased significantly over time. This is as expected under anaesthesia. However, the average decrease was about 1°C over 150 minutes in all ponies, which was clinically acceptable.

Recovery remains a critical phase in equine anaesthesia. In the present study all ponies had good recoveries with minimal or no ataxia, despite the fact the animals did not receive additional sedation before the recovery period. Oxygen was supplemented during the recovery period at a flow rate of 8 L/min via the endotracheal tube and nasally after extubation. Although switching from oxygen to room air is normally well tolerated by most horses, it was deemed prudent in these ponies to supplement oxygen during recovery because of the low PaO_2 values measured during anaesthesia (Hubbell 2005).

In conclusion, the results of this study demonstrated that dexmedetomidine CRIs administered during isoflurane anaesthesia caused statistically significant cardiopulmonary effects typical of α_2 -agonists. However, at the doses used, changes were small and within acceptable clinical range, despite isoflurane administration being at $>$ one MAC. Further studies are justified to evaluate the analgesic properties and anaesthetic-sparing effects of both doses of a dexmedetomidine CRI, and also the cardiopulmonary status of horses when such CRIs are administered together with the minimal dose of isoflurane necessary to allow surgery.

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Chapter 1

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Chapter 2

Influence of a constant rate infusion of dexmedetomidine on cardiopulmonary function and recovery quality in isoflurane anaesthetized horses

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Abstract

Objective To investigate the influence of a dexmedetomidine constant rate infusion (CRI) in horses anaesthetized with isoflurane.

Study design Prospective, randomized, blinded, clinical study.

Animals Forty adult healthy horses (weight mean $491 \pm$ SD 102 kg) undergoing elective surgery.

Methods After sedation [dexmedetomidine, 3.5 μ g/kg intravenously (IV)] and induction IV (midazolam 0.06 mg/kg, ketamine 2.2 mg/kg), anaesthesia was maintained with isoflurane in 55-60% oxygen. Horses were ventilated and dobutamine was administered when hypoventilation [arterial partial pressure of CO₂ > 8.00 kPa (60 mmHg)] and hypotension (mean arterial pressure < 70 mmHg) occurred respectively. During anaesthesia, horses were randomly allocated to receive a CRI of dexmedetomidine (1.75 μ g/kg/hour) (D) or saline (S). Monitoring included end-tidal isoflurane concentration, cardiopulmonary parameters, and need for dobutamine and additional ketamine. All horses received 0.875 μ g/kg dexmedetomidine IV for the recovery period. Age and weight of the horses, duration of anaesthesia, additional ketamine and dobutamine, cardiopulmonary data (ANOVA), recovery scores (Wilcoxon Rank Sum Test), duration of recovery (t-test) and attempts to stand (Mann-Whitney test) were compared between groups. Significance was set at $p < 0.05$.

Results Heart rate (HR) and arterial partial pressure of oxygen were significantly lower in group D compared to group S. An interaction between treatment and time was present for cardiac index, oxygen delivery index and systemic vascular resistance (SVR). End-tidal isoflurane concentration and HR significantly increased over time. Packed cell volume, systolic, diastolic and mean arterial pressure, arterial oxygen content, stroke volume index and SVR significantly decreased over time. Recovery scores were significantly better in group D, with fewer attempts to stand and significantly longer times to sternal position and first attempt to stand.

Conclusions and clinical relevance A dexmedetomidine CRI produced limited cardiopulmonary effects, but significantly improved recovery quality.

Introduction

Inhalation anaesthesia is frequently used in long surgical procedures in horses and carries a higher risk of mortality compared with humans and small domestic animals (Johnston et al. 2002). Since many causes of peri-anaesthetic death can be a consequence of cardiovascular depression, combination anaesthetic protocols are often used to reduce the required amount of inhalants and the associated cardiovascular effects (Steffey & Howland 1978) in an attempt to achieve 'balanced anaesthesia'. Currently, balanced anaesthesia has reached new dimensions with the use of inhalation anaesthetics in combination with short-acting anaesthetic adjuvants. The theory is that the combination of different anaesthetics will act synergistically regarding to desired effects, but not with respect to side-effects (Tonner 2005).

Combination anaesthesia in horses aims at maintaining good intraoperative cardiopulmonary function and minimizing the pain associated with surgery, both of which should result in calmer and more coordinated recoveries (Bettschart-Wolfensberger & Larenza 2007). The use of lidocaine (Doherty & Frazier 1998), ketamine (Muir & Sams 1992) and various α_2 -agonists (Wagner et al. 1992; Neges et al. 2003; Kuhn et al. 2004; Devisscher et al. 2010; Schauvliege et al. 2011) as constant rate infusions (CRIs) have been described in clinical and in experimental equine anaesthetic procedures. These CRIs were reported to provide a sufficient level of analgesia, an increase in anaesthetic depth and a reduction of the minimum alveolar concentration (MAC) of the inhalation agents (Steffey & Pascoe 1991; England & Clarke 1996; Bettschart-Wolfensberger et al. 2001).

Alpha₂-agonists are used frequently in combination anaesthetic protocols in horses, although when these agents are used for sedation, especially after bolus administration, classic side effects including bradycardia, arrhythmias, decreases in cardiac output (\dot{Q}_t) and increases in systemic vascular resistance (SVR) have been documented extensively (England & Clarke 1996; Yamashita et al. 2000). In contrast, cardiac output indexed to weight (CI) and systemic vascular resistance indexed to weight (SVRI) did not differ from pre-sedation values under steady state conditions when medetomidine was used as a CRI in ponies (Bettschart-Wolfensberger et al. 1999). Furthermore, recovery quality was significantly better when horses undergoing

elective surgery received a medetomidine CRI during isoflurane anaesthesia compared to a lidocaine CRI (Ringer et al. 2007).

Dexmedetomidine, the dextro-rotary and active enantiomer of the racemic mixture medetomidine (Vickery & Maze 1989), has marketing authorisation in small animal practice and is one of the most potent and selective α_2 -agonist commercially available. Results of previous studies showed that dexmedetomidine offers some sedative and analgesic benefits over racemic medetomidine in dogs (Kuusela et al. 2000, 2001). Furthermore, dexmedetomidine was reported to have a shorter half-life compared to medetomidine in both dogs (Kuusela et al. 2000) and ponies (Bettschart-Wolfensberger et al. 2005). Dexmedetomidine also significantly reduces the MAC of isoflurane in humans (Aantaa et al. 1997) and dogs (Pascoe et al. 2006).

In ponies, dexmedetomidine was shown to be redistributed more rapidly than in humans due to a larger volume of distribution (Bettschart-Wolfensberger et al. 2005). Consequently, dexmedetomidine has been suggested to be an ideal agent for CRIs in equine anaesthesia. Preliminary studies in experimental ponies in our clinic (Marcilla et al. 2010) showed that two different CRI rates, 1 and 1.75 $\mu\text{g}/\text{kg}/\text{hr}$, after sedation with dexmedetomidine (3.5 $\mu\text{g}/\text{kg}$), caused statistically significant cardiopulmonary effects typical of α_2 -agonists, although cardiovascular function remained within clinically acceptable limits. However, the arterial partial pressure of oxygen (PaO_2) recorded in those anaesthetized ponies, had a tendency to be low. To date, dexmedetomidine has been used under clinical conditions as a CRI in dogs (Uilenreef et al. 2008) and in small ruminants (Kästner et al. 2007), but not in horses.

In our clinic, we have investigated the effects of a CRI of romifidine (Devisscher et al. 2010) and of detomidine (Schauvliege et al. 2011) when given to anaesthetized horses. The aim of this current study was to evaluate the cardiopulmonary and possible isoflurane sparing effects and the influence on recovery quality of a dexmedetomidine CRI of 1.75 $\mu\text{g}/\text{kg}/\text{hr}$ in healthy anaesthetized horses undergoing elective surgery in which anaesthesia is being maintained with isoflurane.

Materials and methods

The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine of the University of Ghent (2009/22).

Animals and instrumentation

Forty adult client-owned horses [American Society of Anesthesiologists (ASA) category I or II] aged between seven months and sixteen years old, weighing 491 ± 102 kg (mean \pm SD), which had been referred for elective surgery (soft tissue or orthopaedic procedures) lasting more than one hour, were included in the study. Horses undergoing head or neck surgery were excluded because of difficulty in evaluation of clinical parameters related to anaesthetic depth.

The horses were assigned randomly to group D (dexmedetomidine) or group S (saline). Food but not water was withheld for twelve hours before general anaesthesia. Pre-anaesthetic examinations were performed the evening before the surgical procedure. All the anaesthetic procedures were performed by the same investigator (MGM) who was unaware of the treatment given.

Dexmedetomidine ($3.5 \mu\text{g}/\text{kg}$, IV) (Dexdomitor, Pfizer Animal Health, Belgium) was given for sedation, after a 12-gauge \times 80mm (Intraflon 2, Ecoen, France) or a 14-gauge \times 55 mm (Vasocan Braunüle Luerlock, B. Braun Melsungen AG, Germany) catheter was placed in the jugular vein. If the horse was not adequately sedated, an additional dose of dexmedetomidine (between 1/4 or 1/2 of the initial dose) was given before induction of anaesthesia. Seven to ten minutes following administration of the sedative dose of dexmedetomidine, anaesthesia was induced with midazolam ($0.06 \text{ mg}/\text{kg}$, IV) (Dormicum, Roche, Belgium) and ketamine ($2.2 \text{ mg}/\text{kg}$, IV) (Anesketin, Eurovet, Belgium) together in the same syringe.

After tracheal intubation (24-30 mm OD tracheal tube, Willy Rusch AG, Germany), the horses were hoisted onto a surgical table covered with soft foam rubber pillows (twenty cms), and positioned as required for the planned surgical procedure. The endotracheal tube was connected to a large animal anaesthetic unit (Matrx medical Inc., NY, USA mounted on a Sulla 909V, Dräger, Germany) with an out-of-circuit vaporizer (Drägerwerk AG, Germany) and a large animal ventilator (Smith respirator LA 2100, model 2002, Veterinary Technics/BDO-Medipass, The Netherlands). Connection to the anaesthetic circuit was considered as time 0 (T0). Anaesthesia was maintained with isoflurane (Isoflo, Abbott Laboratories Ltd, UK) in a mixture of oxygen (O_2) and air, so as to maintain the inspired O_2 fraction (FiO_2) between 55 and 60%. Horses placed in lateral recumbency were allowed to breathe spontaneously. If the

arterial partial pressure of carbon dioxide (PaCO_2) was higher than 8 kPa (60 mmHg), PaO_2 lower than 13.3 kPa (100 mmHg) or respiratory rate (RR) lower than 4 breaths/min for more than three minutes in laterally recumbent animals intermittent positive pressure ventilation (IPPV) was applied, using an ‘assisted-controlled’ respiration mode with a tidal volume of 10 mL/kg, peak inspiratory pressure close to 1.96 kPa (20 cmH₂O), inspiratory time of 2.2 seconds and RR close to 8 breaths/min. All parameters were adapted to maintain PaCO_2 between 4.66 and 6.00 kPa (35 and 45 mmHg). Horses placed in dorsal recumbency were ventilated immediately after positioning on the table as previously described. During anaesthesia, anaesthetic depth was adjusted by altering the inspired isoflurane concentration according to assessment of clinical parameters (respiration, cardiovascular parameters and ocular signs). A ketamine bolus was administered to deepen anaesthesia if the horses showed nystagmus or moved.

Arterial access was achieved by catheterization of the facial artery (22-gauge Vasocan Braunüle Luer Lock, B. Braun Melsungen AG, Germany). This was used to obtain arterial blood for analysis, for withdrawal of blood for the lithium dilution \dot{Q}_t measurements, and for invasive measurement of arterial blood pressures. The pressure monitoring system was zeroed at the level of the right atrium.

Inspiratory and expiratory CO_2 , O_2 and isoflurane concentrations were measured using a calibrated, methane-insensitive, multi-gas analyser (Datex Ohmeda, S/5, D-LCC15-03, OR, USA). This monitor was also used to record the ECG (base-apex lead), systolic (SAP), diastolic (DAP), mean arterial pressures (MAP), peripheral arterial saturation by pulse oximetry (probe placed on the tongue) and body temperature using an oesophageal probe.

Cardiac output was measured with the lithium dilution technique (LiDCOplus Haemodynamic Monitor, LiDCO Ltd., UK). A bolus of lithium chloride (4.5 $\mu\text{mol/kg}$) was injected through the jugular venous catheter for each measurement. Haemoglobin concentration was estimated from the packed cell volume (PCV) [$\text{Hb (g/dL)} = 34 \times \text{PCV (L/L)}$; Linton et al. 2000] measured at T15. Intraoperatively, all horses received flunixin meglumine (1.1 mg/kg, IV) (Endoflurin 50, Ecuphar, Belgium) and intramuscular procaine benzylpenicillin (15000 IU/kg) (Pen-30, V.M.D., Belgium).

Experimental design

As soon as the endotracheal tube was connected to the anaesthetic circuit (T0), group D received a CRI of dexmedetomidine (1.75 µg/kg/hr) while group S received a saline CRI of equivalent volume and rate. The syringes were prepared by one of the co-authors, while the main anaesthetist (MGM) was unaware of the treatment. Constant rate infusions were administered using a syringe driver (Ohmeda 9000, BOC Health care, UK) and were maintained until the end of anaesthesia. Lactated Ringer's solution (Ringer Lactate, Vetoflex, Bioluz, France) was administered IV for the duration of anaesthesia at a rate of 10 mL/kg/hr. A urinary catheter was placed in all the horses.

Values for inspiratory and expiratory CO₂, O₂, isoflurane, heart rate (HR), SAP, MAP, DAP, and body temperature were recorded at five minute intervals throughout anaesthesia. Cardiac output was measured and arterial blood samples were collected for immediate analysis at fifteen minute intervals (ABL5, Radiometer, Denmark).

Cardiac output, CI, stroke volume (SV), stroke volume indexed to weight (SVI), arterial content of oxygen (CaO₂) and oxygen delivery index to weight (DO₂I) were calculated using standard formulae as listed in previous work from our laboratory (Schauvliege et al. 2008).

Total doses of additional boluses of ketamine and dobutamine infusion rates were recorded. Dobutamine (Dobutamine EG, NV Eurogenerics, Belgium) was infused to maintain MAP above 70 mmHg, starting at a rate of 0.5 µg/kg/min and then adjusting the rate as required. The administration rate of dobutamine over time was calculated for each horse according to the individual body weight and the duration of anaesthesia.

At the end of the surgical procedure, all horses received 0.875 µg/kg dexmedetomidine, before they were transported to a padded recovery box where they were allowed to recover without assistance. Oxygen was administered (from 8 to 15 L/min depending on the size of the horse) initially through the endotracheal tube and, after extubation, nasally. The endotracheal tube was removed once the horses were able to swallow. Following extubation, the doors of the recovery box were closed, and recoveries were observed from outside via the continuous images sent by a video camera. Extubation time, time to sternal recumbency, time to stand were recorded, and recovery quality scored on a scale of 1-5 (Table 1). All the recoveries were observed continuously and scored by the same blinded anaesthetist (MGM).

Table 1: Scoring system used to grade recoveries.

Score	Description
1	One attempt to stand, no ataxia.
2	One to two attempts to stand, some ataxia.
3	More than two attempts to stand but quiet recovery.
4	More than two attempts to stand, excitation.
5	Severe excitation. Horse injured.

Statistical analysis

Data was tested for normality of distribution by the Kolmogorov-Smirnov test. The age and weight of the horses, duration of anaesthesia and total doses of additional ketamine and required dobutamine were compared between treatment groups using ANOVA.

The duration of anaesthesia did not exceed sixty minutes in several horses, and therefore only the cardiopulmonary data for sixty minutes after anaesthetic induction were analyzed. This analysis used a mixed model analysis of variance with treatment, time and their interaction as fixed effects and horse as random effect. Recovery scores and duration were compared between treatments using a Wilcoxon rank sum test and t-test respectively. A Mann-Whitney test was used to compare the number of attempts to stand in the recovery. For all analyses the significance level was set at 5%. Results are presented as mean \pm SD unless otherwise stated.

Results

Age (for group S and D respectively, 4 ± 4 and 6 ± 4 years) and weight (469 ± 95 and 513 ± 107 kg) of the horses and duration of anaesthesia (97 ± 28 and 105 ± 44 minutes) did not differ statistically between groups. In group S and D, respectively sixteen and thirteen horses were placed in dorsal recumbency while four and seven were positioned in lateral recumbency. All four horses placed in lateral recumbency in group S were able to breathe spontaneously, maintaining PaCO₂ lower than 8 kPa (60 mmHg) during anaesthesia. In contrast, only one of seven horses in group D was able to breathe spontaneously. All other horses were mechanically ventilated. No significant differences between groups in FiO₂ were found.

Eleven horses (three in group S and eight in group D) (27.5%) received an additional dose of dexmedetomidine to obtain an acceptable level of sedation prior to anaesthesia.

Details of surgical interventions are shown in Table 2.

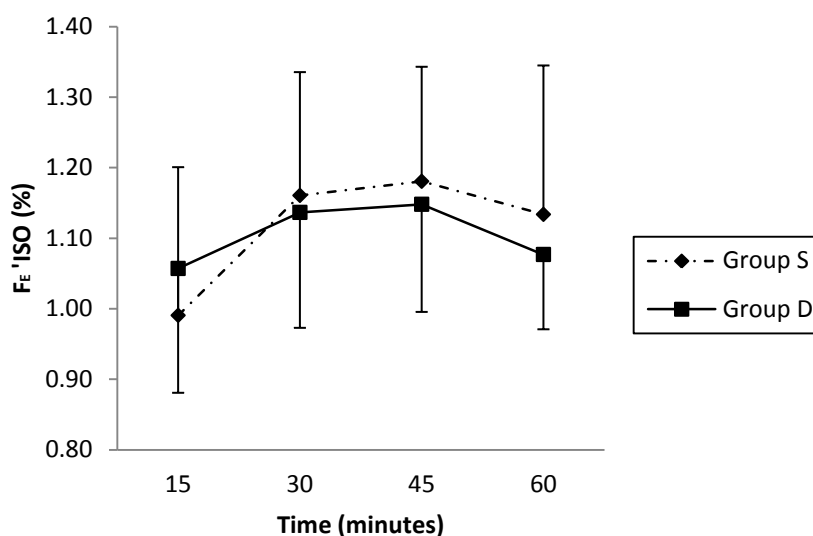
Table 2: Types of surgeries performed in group S (n = 20) and group D (n = 20). Group D received a constant rate infusion of dexmedetomidine (1.75 µg/kg/hr), group S an equivalent volume and rate of saline.

Type of surgery	Group S	Group D
Arthroscopy	11	10
Cryptorchid	3	3
Sarcoid excision	1	2
Umbilical hernia	1	1
Wound	1	1
Sarcoid cryosurgery	0	1
Arthrodesis	0	1
Funiculitis	1	0
Street nail	0	1
Penis amputation	1	0
Varus deformation	1	0

Isoflurane concentrations, ketamine and dobutamine administration (Figure 1)

Overall, the end-tidal isoflurane concentration ($F_{E'}\text{ISO}$) increased over time from ($p = 0.0001$). No significant differences between groups were found (Fig. 1). Twelve horses in group S and eight horses in group D received additional doses of ketamine. The total dose in these horses was 0.82 ± 0.41 and 0.57 ± 0.3 mg/kg for horses in group S and D, respectively ($p = 0.16$). In both groups, seven horses needed one dose of extra ketamine; doses ranging from 0.3 to 1 mg/kg in group S and from 0.3 to 0.9 mg/kg in group D. Two horses in group S needed two doses (0.5 mg/kg), two needed three doses (0.4-0.6 mg/kg) and one horse four extra doses (0.2-0.4 mg/kg). In group D only one horse required a second dose (0.5 mg/kg). Extra doses of ketamine were administered when horses showed nystagmus or moved. Only one horse in each group moved during anaesthesia.

Nine horses in group S (mean dose 0.11 ± 0.06 µg/kg/min) and five horses in group D (mean dose 0.13 ± 0.15 µg/kg/min) received a dobutamine CRI during the anaesthetic period. These mean doses did not differ significantly between groups ($p = 0.6$).



§ Changes over time ($p < 0.05$) were present.

Figure 1: End-tidal isoflurane concentration (F_E'ISO) (in percentage) in forty horses anaesthetized with a standard isoflurane protocol for elective surgery. Horses in group S (n = 20) received a constant rate infusion of saline and horses in group D (n = 20) received dexmedetomidine 1.75 µg/kg/hr.

Cardiopulmonary system (Tables 3-4 & Figures 2-5)

Overall, HR in beats/min was significantly lower ($p = 0.02$) in group D (33 ± 4) compared with group S (37 ± 6) (Fig. 2). An interaction between treatment and time was found for CI ($p = 0.02$) (Fig. 3), DO₂I ($p = 0.02$) (Fig. 4) and SVR ($p = 0.04$). Statistical analysis also showed an increase in HR ($p < 0.0001$) and a decrease in SAP, DAP and MAP ($p < 0.0001$), SVI ($p = 0.003$) and SVR ($p = 0.005$) over time.

Overall, PaO₂ was significantly lower in group D (20 ± 7 kPa; 150 ± 53 mmHg) than in group S (25 ± 9 kPa; 188 ± 68 mmHg) ($p = 0.02$) (Fig. 5). Packed cell volume decreased over time ($p < 0.0001$).

Hypoxaemia (PaO₂ < 8 kPa; < 60 mmHg) occurred only in four horses (three in group S and one in group D), and for short periods of time, mostly after the first hour of anaesthesia.

An interaction between treatment and time was found for DO₂I ($p = 0.02$) (Fig. 4). Arterial oxygen content decreased significantly over time ($p = 0.03$).

Table 3: Cardiovascular parameters (mean ± SD) in forty anaesthetized horses undergoing elective surgery.

Values	Differences	Unit	Group	T15	T30	T45	T60
SAP	§	mmHg	S	119 ± 12	107 ± 10	105 ± 16	107 ± 15
			D	122 ± 17	114 ± 20	107 ± 22	106 ± 20
MAP	§	mmHg	S	95 ± 13	84 ± 11	82 ± 16	86 ± 15
			D	98 ± 17	91 ± 19	86 ± 20	83 ± 19
DAP	§	mmHg	S	81 ± 12	70 ± 11	69 ± 17	72 ± 15
			D	83 ± 15	78 ± 18	73 ± 20	71 ± 18
SVR	§, #	dyne/sec/cm ⁵	S	260 ± 45	209 ± 43	223 ± 86	244 ± 103
			D	263 ± 82	252 ± 76	233 ± 89	223 ± 81
SI	§	mL/kg	S	1.8 ± 0.4	1.9 ± 0.4	1.7 ± 0.4	1.7 ± 0.4
			D	1.9 ± 0.5	1.7 ± 0.5	1.7 ± 0.5	1.7 ± 0.5

Systolic (SAP), mean (MAP) and diastolic (DAP) pressures, systemic vascular resistance (SVR) and stroke index (SI) in forty anaesthetized horses undergoing elective surgeries. Horses in group S (n = 20) received a CRI of saline and horses in group D (n = 20) received dexmedetomidine 1.75 µg/kg/hr.

§ Changes over time (p < 0.05); #Interaction treatment*time (p < 0.05).

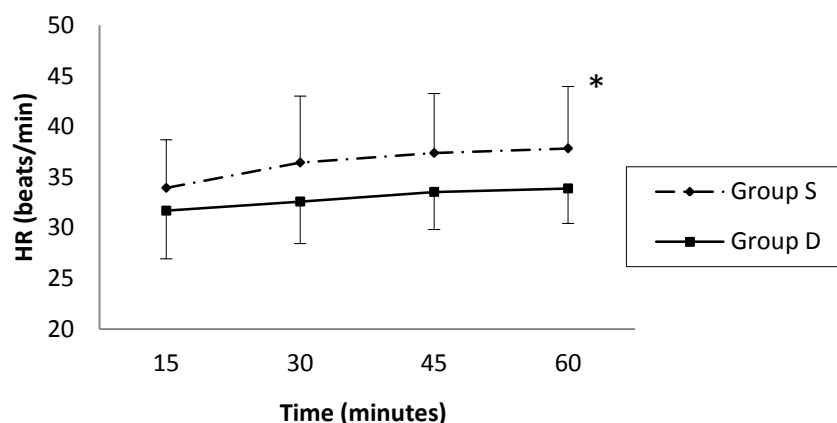
Table 4: Other cardiopulmonary and systemic parameters in forty anaesthetized horses for elective surgery (mean ± SD values).

Values	Differences	Unit	Group	T15	T30	T45	T60
Body temperature	§, #	°C	S	37.1 ± 0.5	37.0 ± 0.6	36.8 ± 0.6	36.8 ± 0.7
			D	37.0 ± 0.4	36.9 ± 0.4	36.8 ± 0.4	36.7 ± 0.4
Arterial pH			S	7.43 ± 0.05	7.43 ± 0.05	7.42 ± 0.06	7.42 ± 0.05
			D	7.41 ± 0.05	7.42 ± 0.05	7.42 ± 0.05	7.41 ± 0.03
Arterial PCO ₂		kPa	S	5.9 ± 0.8	5.9 ± 0.9	6 ± 0.9	6 ± 0.7
			D	6 ± 0.8	6 ± 0.8	6 ± 0.9	6 ± 0.7
Arterial PCO ₂		mmHg	S	44 ± 6	44 ± 7	45 ± 7	45 ± 5
			D	45 ± 6	45 ± 6	45 ± 7	45 ± 5
CaO ₂	§	ml/L	S	143 ± 13	140 ± 11	140 ± 11	140 ± 14
			D	142 ± 17	139 ± 15	137 ± 18	134 ± 17
PCV	§	%	S	30 ± 3	29 ± 3	29 ± 2	29 ± 3
			D	30 ± 3	29 ± 3	29 ± 4	28 ± 3

Body temperature, arterial blood gas results, arterial oxygen content (CaO₂) and packed cell volume (PCV) in 40 anaesthetized horses undergoing elective surgeries.

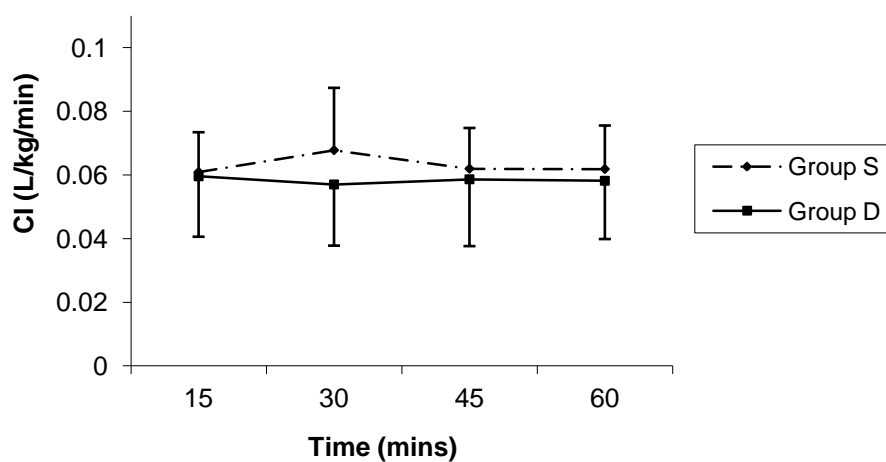
Horses in group S (n = 20) received a constant rate infusion of saline and horses in group D (n = 20) received dexmedetomidine 1.75 µg/kg/hr.

§ Changes over time (p < 0.05); #Interaction treatment*time (p < 0.05).



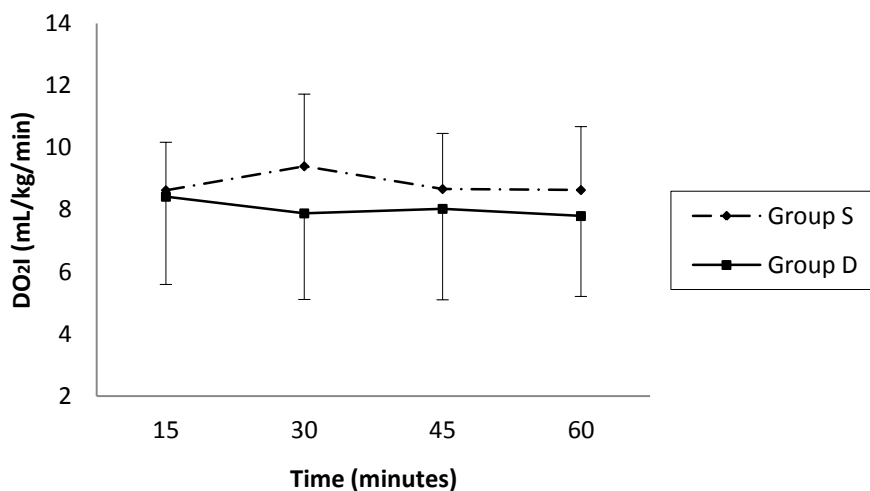
* Significant differences between treatments ($p < 0.05$). A significant increase over time was present in both groups ($p < 0.05$).

Figure 2: Heart rate in forty horses anaesthetized with a standard isoflurane protocol for elective surgery. Horses in group S (n = 20) received a constant rate infusion of saline and horses in group D (n = 20) received dexmedetomidine 1.75 $\mu\text{g}/\text{kg}/\text{hr}$.



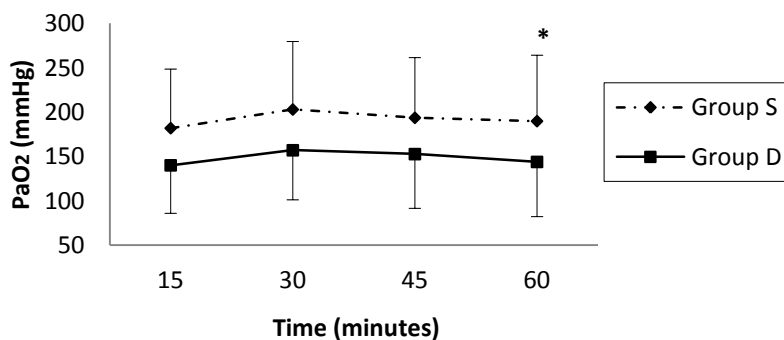
Interaction treatment*time was present ($p < 0.05$).

Figure 3: Cardiac index in forty horses anaesthetized with a standard isoflurane protocol for elective surgery. Horses in group S (n = 20) received a constant rate infusion of saline and horses in group D (n = 20) received dexmedetomidine 1.75 $\mu\text{g}/\text{kg}/\text{hr}$.



Interaction treatment*time was present ($p < 0.05$).

Figure 4: Oxygen delivery index in forty horses anaesthetized with a standard isoflurane protocol for elective surgery. Horses in group S (n = 20) received a constant rate infusion of saline and horses in group D (n = 20) received dexmedetomidine 1.75 µg/kg/hr.



*Significant differences between treatments ($p < 0.05$).

Figure 5: Arterial partial pressure of oxygen in forty horses anaesthetized with a standard isoflurane protocol for elective surgery. Horses in group S (n = 20) received a CRI of saline and horses in group D (n = 20) received dexmedetomidine 1.75 µg/kg/hr.

Recovery scores and times (Tables 5 & 6)

Recovery scores were significantly better in group D ($p = 0.03$) than in group S. Horses in group D needed fewer attempts to stand ($p = 0.04$). Times to sternal recumbency ($p = 0.03$) and first attempt to stand ($p = 0.04$) were significantly longer in group D compared with group S.

Table 5: Recovery scores in forty horses anaesthetized for elective surgery. Horses in group S (n = 20) received a constant rate infusion of saline and horses in group D (n = 20) received dexmedetomidine 1.75 µg/kg/hr.

	Group S	Group D
Score 1	9	15
Score 2	9	6
Score 3	3	0

Significant differences between groups ($p < 0.05$) were found.

Table 6: Recovery times (in minutes) in forty horses anaesthetized for elective surgery. Horses in group S (n = 20) received a constant rate infusion of saline and horses in group D (n = 20) received dexmedetomidine (1.75 µg/kg/hr).

	Differences	Group S	Group D
Extubation time		19 ± 7	21 ± 5
Time to sternal recumbency	*	25 ± 8	31 ± 8
First attempt to stand	*	29 ± 11	36 ± 10
Standing time		32 ± 10	37 ± 11
Extubation to sternal recumbency		6 ± 6	10 ± 7
Sternal to standing time		7 ± 7	6 ± 5

*Significant differences between groups ($p < 0.05$).

Discussion

In the present study, a CRI of dexmedetomidine given to horses during isoflurane anaesthesia had limited cardiopulmonary effects, but failed to reduce the dose of isoflurane required for maintenance. Recovery from anaesthesia was statistically (but not clinically) significantly longer after the dexmedetomidine CRI, but was of better quality.

The design of the present study had limitations, especially to detect anaesthetic-sparing effects of dexmedetomidine. This may have been due to difficulty in correct assessment of depth of anaesthesia. Ringer et al. (2007) reported that, on the basis of the

classical parameters and ocular signs usually used to judge depth of anaesthesia, horses at the 'ideal depth' appear more lightly anaesthetized when a CRI of medetomidine is used. They suggested that horses anaesthetized with medetomidine and assessed as 'light' by the anaesthetist, did not respond to noxious stimuli. In previous studies carried out in our clinic using methods similar to those used here, we failed to demonstrate an isoflurane sparing effect of CRIs of romifidine or detomidine in horses undergoing routine surgical procedures (Devisscher et al. 2010; Schauvliege et al. 2011). An experimental 'MAC reduction study' is required to determine if there are isoflurane sparing effects of dexmedetomidine in horses.

The dose of dexmedetomidine used for pre-anaesthetic sedation of the horses was based on previous studies where 7 µg/kg medetomidine was estimated to be equipotent to 3.5 µg/kg dexmedetomidine (Bettschart-Wolfensberger et al. 2005). In our previous experimental study (Marcilla et al. 2010), this dose produced an adequate level of sedation. However, in the present study, with clinical cases involving different types of horses of varying temperaments, the level of sedation was insufficient in eleven of forty horses and an additional dose of dexmedetomidine was required. Thus slightly higher doses than those used in this study might be preferable in order to obtain acceptable sedation in individual patients, as adequate sedation is essential prior to induction with ketamine. Induction of anaesthesia and subsequent endotracheal intubation were uneventful.

In our previous study, the cardiopulmonary effects of two different CRIs of dexmedetomidine were compared (1 and 1.75 µg/kg/hr) in experimental ponies (Marcilla et al. 2010). The protocol involved a total of 150 minutes of anaesthesia with isoflurane, during which there were periods of infusion of dexmedetomidine at one of the dose rates tested, and periods of 'control' with no dexmedetomidine CRI. Thus the results are not strictly comparable with this current study, which examined only sixty minutes of anaesthesia, involved lower $F_{E} \text{ ISO}$ and included surgical stimulation. In the experimental study, with both administration rates, typical α_2 -agonist associated cardiovascular effects were recorded, but cardiovascular function remained within clinically acceptable limits in all the ponies. However, arterial hypoxaemia was present in four of six ponies. It was considered that this was because of the conformation of the ponies, resulting in alveolar collapse during anaesthesia, causing right-to-left shunting

in the pulmonary circulation (Nyman & Hedenstierna 1989), which has been shown to be more pronounced in fat, round bellied horses (Moens 1989). Cardiovascular function was comparable with both dexmedetomidine administration rates in ponies, possibly due to the occurrence of a 'ceiling effect'. On this basis, the higher of the doses tested was used in the present study, as it was hypothesized that it would provide more sedation, analgesia, and isoflurane sparing effects compared to the lower dose, without causing more pronounced cardiovascular depression.

In this present study, cardiovascular function was well maintained in group D. Overall, HR was significantly lower compared with group S, a typical side effect of α_2 -agonists. The observed decrease in HR was minimal and no periods of severe bradycardia were observed. Only two out of forty horses showed second degree atrio-ventricular blocks, and there was no need for treatment. Heart rate increased over time in both groups, probably due to a gradual waning of the effects of the dexmedetomidine administered for premedication. The cardiopulmonary effects of dexmedetomidine have been shown to be short lasting and should be minimal after thirty minutes, while the plasma concentration should be below the minimal level of detection (0.05 ng/mL) within sixty to ninety minutes (Bettschart-Wolfensberger et al. 2005). Arterial blood pressures (ABPs) decreased over time in both groups, again probably due to a gradually diminishing effect of the dose of dexmedetomidine used for premedication, but also possibly partly due to the increase in FE'ISO that occurred over time, although this increase was small and unlikely to be clinically significant. Although ABP tended to be higher in group D, the difference with group S was not statistically significant. In part, this may be explained by the use of dobutamine to maintain MAP above 70 mmHg in both groups, although the difference in dobutamine used between groups was not statistically significant.

An interaction between treatment and time was found for CI. A peak at T30 occurred in group S, compared to the values at T15 and T45, while CI remained stable for group D. A possible explanation is that surgical intervention usually started close to thirty minutes after anaesthesia induction, and it is possible that the sedation and analgesia provided by the infusion of dexmedetomidine blunted the autonomic response to noxious stimulation. An interaction between treatment and time was also observed for DO₂I (Fig. 4), which changed over time in a similar way as CI, demonstrating the

clear relationship between both variables. In agreement with these findings, the need for additional doses of ketamine tended to be lower and less frequent in horses in group D. It is also interesting to note that although $FE'ISO$ was similar in both groups, CI was not significantly different between treatments, so was not adversely affected by the dexmedetomidine infusion despite the decrease in HR. This contrasts with the results of the experimental studies (Marcilla et al. 2010) in which dexmedetomidine CRI resulted in a significant decrease in CI.

Arterial partial pressure of oxygen was statistically significantly lower in group D compared with group S (Fig. 5), but always in the range to fully saturate haemoglobin, and therefore there was no clinical significance and CaO_2 was comparable in both groups. In conscious horses, α_2 -agonists do cause a small, but usually significant, fall in PaO_2 (England & Clarke 1996), but the cause has not been definitively proven. In the present study most horses were ventilated, so inadequate ventilation was not the cause. Interestingly, all four horses in group S positioned in lateral recumbency were able to maintain $PaCO_2$ below 8 kPa (60 mmHg) and PaO_2 above 13.3 kPa (100 mmHg) without the use of mechanical ventilation. In contrast, in group D, six out of seven laterally recumbent horses needed mechanical ventilation, suggesting that an infusion of dexmedetomidine may reduce respiratory drive in isoflurane anaesthetized horses. A previous study with medetomidine in isoflurane anaesthetized horses (Kalchofner et al. 2006) reported that only three out of 300 horses needed mechanical ventilation. However, in that study mechanical ventilation was only initiated when apnea longer than one minute occurred.

Recovery remains a critical phase in equine anaesthesia. The experimental ponies in our previous study received CRI of dexmedetomidine during anaesthesia, but no additional sedation at the end of anaesthesia. They recovered well with minimal or no ataxia (Marcilla et al. 2010). However, it is possible that there was a 'learning effect', as the ponies had been already anaesthetized for different trials. In the present study, all horses in both groups received dexmedetomidine IV (0.875 μ g/kg) prior the recovery period, this dose being comparable to the 2 μ g/kg of IV medetomidine that has been previously described for use under clinical circumstances (Ringer et al. 2007).

For the evaluation of the recovery scores, the same experienced anaesthetist, unaware of the group, evaluated the recoveries from the video images. Although the

scale used was simple, studies comparing such as scale with more complex scales appeared to have demonstrated that all are equally reliable in assessing recovery quality after general anaesthesia in horses (Vettorato et al. 2010). In the present study recovery was of good quality in both groups, but both had received extra sedation at this time. However, the recovery was scored to be better in group D, the horses having fewer attempts to stand and taking significantly longer times to sternal recumbency and to their first attempt to stand than did horses in group S. Moreover, after extubation horses in group D tended to stay in lateral recumbency for a longer period before going to the sternal position, suggesting that they had recovered from anaesthesia but were more sedated than horses in group S.

In conclusion, the results of the present study demonstrated that a dexmedetomidine CRI at 1.75 $\mu\text{g}/\text{kg}/\text{hr}$, given to healthy isoflurane anaesthetized horses undergoing surgery, failed to reduce the $\text{F}_{\text{E}}\text{I}_{\text{SO}}$ required to maintain anaesthesia under the conditions of this clinical trial, but had no clinically relevant effects on the cardiovascular system. Although PaO_2 was significantly lower in group D, DO_2I did not differ between groups. The horses receiving dexmedetomidine CRI took longer to recover but the recovery quality was better than that of group S. Experimental studies are required to find if, and at what dose, a dexmedetomidine CRI can reduce the MAC of isoflurane in horses.

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Chapter 3

Effects of a constant rate infusion of dexmedetomidine on the minimum alveolar concentration of sevoflurane in ponies

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Summary

Reasons for performing study Dexmedetomidine has been administered in the equine as a constant rate infusion (CRI) during inhalation anaesthesia preserving optimal cardiopulmonary function with calm and coordinated recoveries. Inhalant anaesthetic sparing effects have been demonstrated in other species but not in horses.

Objectives To determine the effects of a CRI of dexmedetomidine on the minimum alveolar concentration (MAC) of sevoflurane in ponies.

Methods Six healthy adult ponies were involved in this prospective, randomized, crossover, blinded, experimental study. Each pony was anaesthetized twice (three-weeks washout period). After induction with sevoflurane in oxygen (via nasotracheal tube), the ponies were positioned on a surgical table (T0) and anaesthesia was maintained with sevoflurane (expired sevoflurane fraction 2.5%) in 55% oxygen. The ponies were randomly allocated to treatment D [intravenous (IV) dexmedetomidine 3.5 µg/kg (T10-T15) followed by a CRI of dexmedetomidine at 1.75 µg/kg/hr] or treatment S (bolus and CRI of saline at the same volume and rate as treatment D). After T60, MAC determination, using a classic bracketing technique, was initiated. Stimuli consisted of constant-current electrical stimuli at the skin of the lateral pastern region. Triplicate MAC estimations were obtained and averaged in each pony. Monitoring included pulse oximetry, electrocardiography, anaesthetic gas monitoring, arterial blood pressure measurement and arterial blood gases. Normocapnia was maintained by mechanical ventilation. Analysis of variance (treatment and period as fixed factors) was used to detect differences between treatments ($\alpha = 0.05$).

Results An IV dexmedetomidine CRI decreased mean \pm SD sevoflurane MAC from $2.42 \pm 0.55\%$ to $1.07 \pm 0.21\%$ (mean MAC reduction $53 \pm 15\%$).

Conclusions and clinical relevance A dexmedetomidine CRI at the reported dose significantly reduces the MAC of sevoflurane.

Introduction

Balanced anaesthetic techniques are often used to reduce the required amount of inhalants and their associated cardiovascular effects (Steffey & Howland 1978). Alpha₂-agonists produce sedation and analgesia in all species and have been shown to reduce inhalational anaesthetic requirements when administered as a bolus (Steffey et al. 2000) or as a constant rate infusion (CRI) in horses (Wagner et al. 1992; Bettschart-Wolfensberger et al. 2001; Neges et al. 2003; Kuhn et al. 2004), although clear minimum alveolar concentration (MAC) reductions were not always reported (Devisscher et al. 2010; Schauvliege et al. 2011; Marcilla et al. 2012). However, their classic side effects in horses (bradycardia, arrhythmias, a decrease in cardiac output and an increase in vascular resistance (England & Clarke 1996; Yamashita et al. 2000) should be considered.

The use of dexmedetomidine, currently the most selective α_2 -agonist agent, has been reported in ponies at the dose of 3.5 $\mu\text{g}/\text{kg}$ (Bettschart-Wolfensberger et al. 2005). Its use as a CRI experimentally in ponies (at rates of 1 and 1.75 $\mu\text{g}/\text{kg}/\text{hr}$) (Marcilla et al. 2010) and clinically in horses (1.75 $\mu\text{g}/\text{kg}/\text{hr}$) (Marcilla et al. 2012) after a sedative dose of 3.5 $\mu\text{g}/\text{kg}$ has also been studied. In ponies, dexmedetomidine has been shown to be a rapidly redistributed and short-acting sedative drug, with a rapid initial decline of the drug concentration, which indicates that plasma levels can be rapidly adjusted to the needs of the patients (Bettschart-Wolfensberger et al. 2005). These characteristics encourage the use of dexmedetomidine as a CRI in equine balanced anaesthetic techniques.

In the present MAC study, sevoflurane was chosen because of its low blood solubility, which facilitates a quick induction and recovery, as well as more rapid changes in anaesthetic depth compared with other volatile agents (Brown 1995). Additionally, sevoflurane has an acceptable smell and induces a smooth induction of anaesthesia without breath holding or signs of airway irritation (Aida et al. 1994). The MAC of sevoflurane in horses has been reported to be $2.31 \pm 0.11\%$ (Aida et al. 1994), $2.42 \pm 0.24\%$ (Rezende et al. 2011) and $2.84 \pm 0.16\%$ (Steffey et al. 2005) in different studies. This volatile agent is presently used in the equine (Matthews et al. 1999; Rezende et al. 2011) although its use is not licensed.

The objective of the present study was to determine the MAC of sevoflurane in anaesthetized ponies when receiving a CRI of dexmedetomidine or a CRI of saline by using a constant-current (CC) noxious stimulation (Levionnois et al. 2009).

Materials and methods

The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine of the University of Ghent (2011/059).

Animals and instrumentation

Six healthy ponies (five geldings and one mare) aged 12.7 ± 2.8 years and weighing 294 ± 51 kg were included in this study. The left carotid artery had been transposed to a subcutaneous (SC) position at least seven years before the experiments. Food (not water) was withheld for twelve hours before anaesthesia.

A 14-gauge intravenous (IV) catheter (Venocan, Kruuse, Denmark) was placed in the left jugular vein following SC administration of 0.5 mL mepivacaine 2% (Scandicaine, Astrazeneca, Belgium). A nasotracheal silicone tube (Endo-Tracheaal Sil V-PET-14/16, Vtrade, Belgium) was inserted into the trachea, via the nose, using lidocaine gel (Xylocaine, Astrazeneca, Belgium) and silicone spray (Silikonspray, Kirchner & Wilhelm GmbH & Co. KG, Germany) to facilitate placement. Preanaesthetic medication was not administered. A rope twitch was placed on the nose during nasotracheal intubation of the less cooperative ponies.

The ponies were manually restrained against the wall of the recovery box by two anaesthetists, while head and tail ropes were used for additional support (another two persons). Cotton was placed inside the ears to reduce auditory stimuli. The nasotracheal tube was connected to a circle system (Large Animal Ventilator Dräger AV, North American Dräger, PA, USA) with a 30 L reservoir bag, using plastic breathing hoses of 3.5 m length and 55 mm internal diameter. Anaesthesia was induced with 5% sevoflurane (Sevorane, Abbott, Belgium) in 8 L/min oxygen. The induction time (time from connection to the anaesthetic machine to lateral recumbency) and expired sevoflurane fraction ($F_E \text{ SEVO}$) at the moment of induction were recorded.

After induction of anaesthesia, the ponies remained in lateral recumbency while inhaling sevoflurane to deepen the plane of anaesthesia, for three to five minutes. They

were then hoisted onto a surgical table covered with soft foam rubber pillows, and positioned in right lateral recumbency. The nasotracheal tube was then connected to a large animal anaesthetic unit (Matrx, Matrx Medical Inc., NY, USA mounted on a Sulla 909V, Dräger, Germany) and a large animal ventilator (Smith respirator LA 2100 model 2002, Veterinary Technics/BDO-Medipass, The Netherlands). Anaesthesia was maintained with sevoflurane in oxygen/air with an inspired oxygen fraction (FiO_2) of 55%. The sevoflurane vaporizer setting was adjusted to maintain an F_E 'SEVO of 2.5% for the first hour of anaesthesia. Respiration mode was 'assisted-controlled', with a tidal volume of 10 ml/kg, respiratory rate of 10 breaths/min, peak inspiratory pressure of 1.96 kPa (20 cmH₂O) and inspiration time of 1.8 seconds. Positive end expiratory pressure (PEEP) of 0.49 kPa (5 cmH₂O) was applied by means of a home-made, plastic, water-filled cylinder, in which the distal end of the expiratory limb was positioned five cm below the water surface. The settings were adjusted to maintain arterial partial pressure of carbon dioxide ($PaCO_2$) between 6.67 and 8.00 kPa (50 and 60 mmHg) .

Lactated Ringer's solution (Hemofiltratie BH 504, Dirinco, The Netherlands) was infused during the whole anaesthetic (3 ml/kg/hr). A urinary catheter was placed.

A 22-gauge catheter (Venocan, Kruuse, Denmark) was placed in the left carotid artery and connected to a pressure transducer (at the level of the right atrium and zeroed to atmospheric pressure) to record arterial blood pressures. Two additional catheters were placed (left facial and metatarsal arteries), and non-invasive blood pressure cuffs on the tail, both metacarpal and right metatarsal arteries for the performance of a parallel study. Blood samples were withdrawn every twenty to thirty minutes from the facial arterial catheter for blood gas analysis [pH, standard base excess (SBE) and arterial partial pressures of oxygen (PaO_2) and carbon dioxide] (ABL5, Radiometer, Denmark). Packed cell volume was determined by centrifugation.

Inspiratory and expiratory carbon dioxide, oxygen and sevoflurane concentrations were continuously measured using a methane-insensitive, multiparameter monitoring device (Datex Ohmeda, S/5, D-LCC15-03, OR, USA), which was calibrated before every procedure (QUICK CAL™ Calibration gas Desflurane, GE Healthcare Finland Oy, Finland) and also used to record the electrocardiogram, systolic, diastolic and mean arterial pressures, peripheral arterial saturation by pulse oximetry (probe on the tongue) and body temperature by a nasal

probe. A warm-water blanket was placed under the ponies (Aquamatic K Thermia Model RK-625, Gorman-Rupp Industries Div., OH, USA) and the room temperature maintained at 23 °C in order to reduce heat loss.

According to our protocol, dobutamine would be infused to maintain the mean arterial pressure > 65 mmHg in case of hypotension (mean arterial pressure < 60 mmHg) and the FiO₂ would be increased if hypoxaemia occurred (PaO₂ < 60 mmHg), but these measures were not needed in any of the ponies.

Experimental design

Each pony was anaesthetized twice and was administered treatment S (saline) or D (dexmedetomidine), with a three weeks washout period. The order of the treatments was randomized.

Once the ponies were positioned on the table (T0), anaesthesia was maintained for sixty minutes (T60) with sevoflurane at an F_E'SEVO of 2.5%. At T10, the ponies received an IV bolus of dexmedetomidine (3.5 µg/kg) (Dexdomitor, Orion Corporation, Finland) (treatment D) or a bolus of saline at the same volume (treatment S) over a period of five minutes (T10-T15). From T15 onwards, a CRI of dexmedetomidine was administered (1.75 µg/kg/hr) (treatment D) or a saline CRI at the same volume and rate (treatment S), until the end of the anaesthesia.

Determination of MAC

A CC electrical stimulation was used for MAC determination (Levionnois et al. 2009). Two electrodes (Neuroline 70005-J/12, Ambu GmbH, Germany) were placed on the shaved and degreased skin of the lateral aspect of the distal pastern region of the left forelimb with an interelectrode distance of one cm. Both electrodes were connected to an electrical stimulator (Grass S88, Grass Medical Instruments, MA, USA) equipped with a CC unit (Grass Constant Current Unit, Grass Technologies, RI, USA) to ensure delivery of CC stimuli despite possible variations in interelectrode resistance. The stimuli consisted of a twenty five millisecond train of five one millisecond CC (forty milliamperes) square-wave pulses. The trains of five were delivered at a frequency of 5 hertz. The resistance between the electrodes was measured before each stimulation using a multimeter (Digital multimeter, VC260, Voltcraft, Conrad Electronic SE,

Germany). If it increased above three kilohms, the electrodes were replaced to ensure discharge of a current of forty milliamperes. Further details of the equipment used have been previously reported (Spadavecchia et al. 2003).

After sixty minutes of anaesthesia using 2.5% $F_E'SEVO$, the first CC noxious stimulus was applied. The response to electrical stimulation was evaluated by the main anaesthetist (MGM), who was unaware of the treatment and was not allowed to observe the monitored variables (only clinical assessment of anaesthetic depth). The response to the electrical stimuli was considered positive when a gross purposeful movement of head, limbs or tail occurred and/or swallowing or generalized muscle tremors were observed following a CC noxious stimulus. Gross purposeful movement without CC stimulation was also considered a positive response. A negative response was where none of the described movements occurred, whereas nystagmus and changes to physiological parameters were considered as negative responses as well. If the reaction was negative, the $F_E'SEVO$ was decreased by 0.2% and maintained for twenty minutes before a further stimulation. If the response was positive, the $F_E'SEVO$ was increased by 0.2% and maintained for twenty minutes prior to further stimulation. The MAC was determined as the average of the lowest concentration preventing a positive response and the highest concentration allowing a positive response. After the first MAC estimation the $F_E'SEVO$ was again increased or decreased in steps of 0.2% (second estimation of MAC value). For the third estimation of MAC, increases or decreases of $F_E'SEVO$ were made in steps of 0.1%.

Each MAC determination was corrected to standard atmospheric pressure. The final MAC value was determined as the mean of the three estimations. Local tetracycline spray (Chlortetra Spray, Eurovet, Belgium) was applied to the stimulated area at the end of the experiment. No skin lacerations or wounds were noticed.

Recording of cardiopulmonary variables and arterial blood gases

Cardiopulmonary values were recorded every ten minutes, while arterial blood gases were determined every twenty-thirty minutes. The mean of the three values recorded before each MAC estimation (i.e. recorded immediately before each electrical stimulation for first, second and third MAC estimations) was additionally calculated.

Recovery period

After determination of the third MAC value and once the ponies were breathing spontaneously, they received a bolus of dexmedetomidine (0.875 µg/kg) and were transported to a padded recovery box. Oxygen was insufflated (8 L/min) through the nasotracheal tube and nasally after extubation. Intranasal phenylephrine (Phenylephrine 10%, Théa Pharma, Belgium) was administered into both nostrils whilst still intubated to reduce post-anaesthetic upper airway obstruction. After swallowing, the nasotracheal tube was removed. The ponies were allowed to recover with manual support of the tail. Extubation time, time to sternal recumbency and time to stand were recorded. Recoveries were scored as previously described (Marcilla et al. 2010) by the main anaesthetist, who was unaware of the administered treatment.

Data analysis

Analysis of variance with treatment and period as fixed factors was used to detect differences between treatments ($\alpha = 0.05$). Correctness of the model was confirmed by analysis of the residuals: the Kolmogorov-Smirnov test indicated a normal distribution of the standardized residuals, while equality of variances and absence of outliers in the data set were confirmed using a scatterplot of the studentized residuals versus the predicted values.

Results

Placement of the nasotracheal tube was possible in all ponies without sedation within two to three minutes with minimal resistance, although a nose twitch was needed in some of them. Inflation of the cuff, lubricated with lidocaine gel, initially resulted in a few (1-3) attempts to cough, but was well tolerated afterwards.

Induction of anaesthesia was smooth with minimal struggling in five of the six ponies. Mean induction time in those ponies was 8.8 ± 2.3 minutes after connection to the anaesthetic machine, while mean \pm SD F_E 'SEVO values of $2.12 \pm 0.14\%$ were required to achieve lateral recumbency.

Pony number 3, a relatively nervous female, panicked and struggled when signs of ataxia occurred. For safety reasons, 1 mg/kg xylazine (Xyl-M, VMD, Belgium) was administered IV. After this, induction of anaesthesia was uneventful. The bolus of the

test drug was administered forty minutes after xylazine in order to avoid possible interferences with MAC determination. The remainder of the anaesthesia was the same as in the other ponies, with a first stimulus forty five minutes after administration of the drug bolus. The same sedative protocol was used for the second treatment in that pony.

Determination of MAC (Table 1)

Table 1 includes the individual MAC values for treatment S and D. The sevoflurane MAC values (mean \pm SD) of treatment D ($1.07 \pm 0.21\%$) were significantly lower compared to treatment S ($2.42 \pm 0.55\%$). The sevoflurane MAC reduction induced by the dexmedetomidine CRI ranged between 34 and 79%, with a mean value of $53 \pm 15\%$.

From the thirty six MAC estimations (three per pony), fourteen were negative responses to stimuli and twenty two were positive responses (fifteen were clear, purposeful movements in response to the electrical stimuli or the inflation of a non-invasive blood pressure cuff and the remaining seven positive reactions were considered spontaneous movements).

Table 1: Individual (mean and range) and overall mean (\pm SD) sevoflurane minimum alveolar concentration (MAC) values (%) and percentage reduction of sevoflurane MAC.

Pony	Treatment S	Treatment D	Percentage MAC Reduction
1	3.52 (3.48-3.53)	0.74 (0.71-0.76)	79
2	2.21 (1.8-2.44)	0.99 (0.96-1.01)	55
3*	2.34 (2.17-2.62)	1.02 (1-1.05)	56
4	2.21 (2.2-2.25)	1.06 (0.96-1.11)	52
5	2.23 (2.22-2.27)	1.28 (1-1.45)	43
6	1.99 (1.96-2.01)	1.32 (1.15-1.4)	34
Mean \pm SD	2.42 ± 0.55	1.07 ± 0.21	53 ± 15

Ponies received either a bolus of saline followed by a constant rate infusion (CRI; treatment S) or a bolus of dexmedetomidine followed by a CRI (treatment D).

*Pony 3 received 1 mg/kg bwt xylazine (IV) prior to induction.

Cardiopulmonary system and body temperature (Table 2)

Throughout anaesthesia, cardiopulmonary function was well maintained within acceptable limits in all the ponies receiving both treatments.

Table 2: Cardiopulmonary parameters, body temperature and duration of anaesthesia in six sevoflurane anaesthetized ponies.

Variable	Unit	Treatment S	Treatment D
Heart rate	beats/min	42 ± 5	36 ± 3
Systolic arterial pressure	mmHg	141 ± 15	104 ± 7
Mean arterial pressure	mmHg	114 ± 8	83 ± 4
Diastolic arterial pressure	mmHg	94 ± 6	69 ± 4
Arterial pH	-	7.36 ± 0.04	7.42 ± 0.02
Standard base excess	mmol/L	5 ± 3	9 ± 1
PaCO ₂	kPa	7.2 ± 1	7 ± 1
	mmHg	54 ± 4	52 ± 4
PaO ₂	kPa	14.3 ± 6.1	15.9 ± 5.7
	mmHg	107 ± 46	119 ± 43
Body temperature	°C	37.0 ± 0.7	36.4 ± 0.6
Duration of anaesthesia	min	178 ± 32	330 ± 32

Ponies received either a bolus of saline followed by a CRI (treatment S) or a bolus of dexmedetomidine followed by a CRI (treatment D). Values were obtained at the time of MAC determination (mean±SD).

Duration of anaesthesia (Table 2) and recovery (Table 3)

Total anaesthesia times (mean ± SD) were significantly shorter for treatment S (178 ± 32 minutes) compared with treatment D (330 ± 32 minutes).

Recovery qualities for all twelve anaesthetic procedures were graded as score 1 (1 attempt to stand, no ataxia). Mean (± SD) extubation time (treatment S 8 ± 4 versus treatment D 9 ± 4 minutes), time to sternal recumbency (treatment S 22 ± 5 versus treatment D 23 ± 10 minutes) and time to stand (treatment S 29 ± 4 versus treatment D 27 ± 9 minutes) were comparable between treatments.

Table 3: Extubation, sternal and standing times (in minutes) and number of attempts to stand in six anaesthetized ponies.

Pony	Treatment	Extubation Time	Time to sternal	Time to stand	Attempts to stand
1	S	5	20	30	1
1	D	7	13	23	1
2	S	15	27	32	1
2	D	8	33	38	1
3*	S	7	29	30	1
3*	D	15	30	30	1
4	S	2	17	32	1
4	D	7	10	15	1
5	S	10	20	22	1
5	D	5	19	20	1
6	S	7	20	25	1
6	D	12	30	35	1

Ponies received either a bolus of saline followed by a constant rate infusion (CRI; treatment S) or a bolus of dexmedetomidine followed by a CRI (treatment D). *Pony 3 received 1 mg/kg bwt xylazine (IV) prior induction.

Discussion

The results of this study show that a dexmedetomidine bolus followed by a CRI of dexmedetomidine at the reported doses significantly reduces the MAC of sevoflurane (mean \pm SD reduction of $53 \pm 15\%$), although individual differences were found.

Induction of anaesthesia by halothane (Bennett et al. 2004), isoflurane (Steffey et al. 2000), sevoflurane (Aida et al. 1994) and desflurane (Tendillo et al. 1997) via face mask has been used in MAC studies in the equine in order to avoid the possible impact of other drugs. To avoid pollution of the working place and to minimize struggling with the ponies, nasotracheal intubation was preferred over the mask induction technique. To the authors' knowledge this technique has never been described for MAC studies in the equine.

In the present study, stepwise (0.2%) increases or decreases in $FE'SEVO$ were quickly obtained, minimizing the duration of anaesthesia. The new level of $FE'SEVO$ was maintained for twenty minutes before the next stimulus was applied. This period was shorter than in most similar studies, so it might be hypothesized that the interval was too short to assure a good agreement between the end-tidal and arterial sevoflurane concentration. However, the difference between the inspiratory and expiratory sevoflurane concentrations was never higher than 10%, suggesting that the difference

between the end-tidal and arterial sevoflurane concentration was minimal. Indeed, factors which produce errors in the estimation of arterial anaesthetic partial pressure from end-tidal pressure analysis usually also contribute to large inspired to end tidal differences (Eger & Bahlman 1971).

Five of the six ponies tolerated the assisted induction technique quite well. One relatively nervous female pony panicked severely once she became ataxic during the induction of anesthesia. In order to reduce the risk for both the pony and the investigators, xylazine was administered. Once a good level of sedation was achieved, induction with sevoflurane by the nasotracheal tube was performed without problems. Xylazine was chosen due to its short duration of action (Yamashita et al. 2000), although dexmedetomidine would also have been a viable alternative (Bettschart-Wolfensberger et al. 2005). To further reduce any influence of the administered sedative drug in that pony, the application of the first stimulus was delayed ninety minutes after the xylazine administration, a time sufficient to allow the MAC sparing effect of xylazine to have worn off (Steffey et al. 2000).

The MAC of an inhaled anaesthetic has been defined as the concentration required to prevent gross movement in response to a defined supramaximal noxious stimulus and has been used to compare the potencies of volatile anaesthetic agents (Eger et al. 1965). The MAC is affected by multiple factors (Eger et al. 1965; Regan & Eger 1976; Yamashita et al. 2009), such as differences in MAC determination methodology [type of stimulus (Steffey et al. 2000; Levionnois et al. 2009), place of stimulation (Doherty & Frazier 1998; Steffey et al. 2000; Bettschart-Wolfensberger et al. 2001) or definition of responses (Aida et al. 1994)], which may be responsible for the collection of different MAC values in different studies. To avoid influences in the present study, the ponies of the same bodyweight and age were used in a crossover study design and premedication was avoided (only one pony required sedation). Normocapnia was maintained by using mechanical ventilation, while PEEP and a warm-water blanket were used in order to reduce the risk of hypoxaemia (Wilson & McFeely 1991; Moens & Böhm 2011) and hypothermia respectively. Although the experiment was performed near sea level, a correction to standard atmospheric pressure was made to compensate for possible differences in ambient pressure.

The most common stimulus in equines is an electrical stimulation of the oral mucosal membranes (Aida et al. 1994; Tendillo et al. 1997; Steffey et al. 2000; Rezende et al. 2011). A similar stimulus has also been described over the palmar digital nerve using subcutaneous electrodes (Doherty & Frazier 1998) and at the coronary band by acupuncture needles (Bettschart-Wolfensberger et al. 2001). Quite recently, CC surface-electrode stimulation was shown to result in more repeatable MAC estimations and clearer reactions compared with two constant-voltage stimuli (Levionnois et al. 2009). In the present study, the surface electrodes were placed on the shaved and degreased skin over the lateral aspect of the distal pastern region of the left forelimb. All MAC determinations per pony and per session were close together, confirming the reportedly high repeatability of MAC determinations using the CC stimulation technique.

In most studies, only gross, purposeful movements induced by a stimulus are accepted as positive responses (Doherty & Frazier 1998; Bennett et al. 2004). In the present experiment, nystagmus and physiological parameter modifications, such as increases in heart rate and arterial blood pressure, were not considered as positive reactions, but in contrast with most MAC studies, swallowing, muscle tremors and any movements of head, limbs, ears or tail were all considered as positive responses. No attempts were made to distinguish between purposeful movements and a classic withdrawal reflex, because the distinction between them can be subtle and is often subjective. Moreover, withdrawal reflexes, swallowing, etc. were considered as positive responses because they are not tolerated under clinical circumstances either. The objective of the present study was to determine the MAC at which a surgical depth of anaesthesia is reached. This classification of the responses to electrical stimuli was similar to the one used by Aida et al. (1994), who rated the responses on a two-point scale, positive or negative.

The MAC value of sevoflurane during treatment S was $2.42 \pm 0.55\%$, which is similar to that reported in horses (Aida et al. 1994; Steffey et al. 2005; Rezende et al. 2011), despite the use of a different definition of MAC. In order to reduce the amount of volatile anaesthetics used and to minimize their cardiovascular depressant effects, several MAC studies with different α_2 -agonists have been performed in horses. Single boluses of xylazine reduced the MAC of isoflurane (Steffey et al. 2000) and halothane (Bennett et al. 2004). A romifidine CRI significantly reduced the expiratory isoflurane

concentration in one clinical study (Kuhn et al. 2004), whereas another failed to find a clear reduction of the anaesthetic requirement (Devisscher et al. 2010). Detomidine reduced the MAC of halothane by 33% when infused continuously (Wagner et al. 1992), although no reduction was found in isoflurane anaesthetized horses (Schauvliege et al. 2011). A medetomidine CRI reduced the MAC of desflurane by 28% in experimental ponies (Bettschart-Wolfensberger et al. 2001) and about 20% in isoflurane anaesthetized clinical horses (Neges et al. 2003).

A bolus of dexmedetomidine followed by a CRI failed to reduce the isoflurane requirements in our previous clinical study (Marcilla et al. 2012). In contrast, a very clear and significant MAC reduction was found here. It is interesting to note that all of the studies in which no difference in the end-tidal anaesthetic agent concentration could be demonstrated, were blinded clinical trials (Devisscher et al. 2010; Schauvliege et al. 2011; Marcilla et al. 2012). A possible reason might be that horses receiving infusions of α_2 -agonists at the 'ideal depth' appeared to be more lightly anaesthetized on the basis of the classical parameters and ocular signs used to judge depth of anaesthesia (Ringer et al. 2007). Consequently, it becomes difficult to detect reductions in volatile anaesthetic requirements if the anaesthetist is unaware of the treatment and evaluates anaesthetic depth based on clinical parameters (Marcilla et al. 2012).

Throughout anaesthesia, cardiopulmonary function and arterial blood gases were clinically acceptable. Further studies are necessary to evaluate the effects of dexmedetomidine in addition to sevoflurane in comparison with pure sevoflurane anaesthesia at the respective MAC levels, with detailed cardiopulmonary assessment. Values before MAC estimation, as shown in table 2, are not compared statistically between both treatments as duration of anaesthesia was significantly longer during treatment D and cardiopulmonary function may change over time (Gasthuys et al. 1990; Steffey et al. 1990).

In order to avoid potential hypoxaemia, mechanical ventilation combined with PEEP was applied (Wilson & McFeely 1991; Moens & Böhm 2011), while an FiO_2 of 55% was maintained to avoid atelectasis (Marntell et al. 2005). Although the PaO_2 values were still lower than theoretically expected, the mean arterial PaO_2 was higher than 13.3 kPa (100 mmHg) in both treatments, and individual PaO_2 values were never below 8 kPa (60 mmHg).

All the ponies received a low dose of dexmedetomidine before the recovery and, despite a rather long duration of anaesthesia, all recoveries were very good and uneventful. Differences in recovery times and qualities were not found in this study, probably because these ponies had been anaesthetized previously and were used to recover from anaesthesia.

In conclusion, dexmedetomidine administered as a bolus followed by a CRI at the dose and rate reported here significantly decreased the MAC of sevoflurane by $53 \pm 15\%$ (mean \pm SD).

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Chapter 4

Influence of dexmedetomidine on the minimum end-tidal sevoflurane concentration necessary to prevent movement during a constant rate infusion of morphine in ponies

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Abstract

Objective To compare the effects of a constant rate infusion (CRI) of dexmedetomidine and morphine to those of morphine alone on the minimum end-tidal sevoflurane concentration necessary to prevent movement (MAC_{NM}) in ponies.

Study design Prospective, randomized, crossover, blinded, experimental study.

Animals Five healthy adult gelding ponies were anaesthetised twice with a three weeks washout period.

Methods After induction of anaesthesia with sevoflurane in oxygen (via nasotracheal tube), the ponies were positioned on a surgical table (T0), and anaesthesia was maintained with sevoflurane ($F_E SEVO$ 2.5%) in 55% oxygen. Monitoring included pulse oximetry, electrocardiography and measurement of anaesthetic gases, arterial blood pressure and body temperature. The ponies were mechanically ventilated and randomly allocated to receive IV treatment M [morphine 0.15 mg/kg (T10-T15) followed by a CRI (0.1 mg/kg/hr)] or treatment DM [dexmedetomidine 3.5 μ g/kg plus morphine 0.15 mg/kg (T10-T15) followed by a CRI of dexmedetomidine 1.75 μ g/kg/hr and morphine 0.1 mg/kg/hr]. At T60, a stepwise MAC_{NM} determination was initiated using constant current electrical stimuli at the skin of the lateral pastern region. Triplicate MAC_{NM} estimations were obtained and then averaged in each pony. Analysis of variance was used to detect differences between treatments ($\alpha = 0.05$).

Results Sevoflurane-morphine MAC_{NM} was $2.79 \pm 0.73\%$. The addition of a continuous infusion of dexmedetomidine significantly reduced sevoflurane MAC_{NM} to $0.89 \pm 0.22\%$ (mean MAC_{NM} reduction $67 \pm 11\%$).

Conclusion and clinical relevance Co-administration of dexmedetomidine and morphine CRIs significantly reduced the MAC_{NM} of sevoflurane compared with a CRI of morphine alone at the reported doses.

Introduction

Morphine is a μ -opioid receptor agonist that has been used during equine anaesthesia mainly to provide analgesia. Its use has been considered controversial due to the potential occurrence of dangerous behaviour, cardiopulmonary disturbances, respiratory depression in anaesthetised horses (Steffey et al. 2003) and a reduction of gastrointestinal motility (Roger et al. 1985). However, clinical studies reported minimal haemodynamic and ventilatory changes (Mircica et al. 2003; Clark et al. 2005), improvement of the recovery qualities (Mircica et al. 2003; Love et al. 2006; Clark et al. 2008) and no increased incidence of post surgical colic (Mircica et al. 2003).

With regard to the effects of morphine on anaesthetic agent requirements, horses undergoing elective surgical procedures receiving a morphine constant rate infusion (CRI) tended to receive fewer and lower doses of additional anaesthetic drugs (Clark et al. 2005). In contrast, IV boluses of morphine at two different doses increased, decreased or did not change the minimum alveolar concentration (MAC) of isoflurane in anaesthetized horses (Steffey et al. 2003). The influence of a morphine infusion on the MAC of volatile agents has not been reported in horses.

Alpha₂-agonists are often combined with opioids in standing horses to achieve neuroleptoanalgesia, producing synergistic analgesic effects (Clarke & Paton 1988), resulting in reliable sedation and stable cardiorespiratory function (Solano et al. 2009). However, under general anaesthesia, the concurrent IV bolus administration of two doses of morphine failed to further reduce the MAC of halothane compared to xylazine alone in adult horses (Bennett et al. 2004). An IV dexmedetomidine CRI decreased the mean \pm SD MAC of sevoflurane in ponies from 2.42 ± 0.55 to $1.07 \pm 0.21\%$ (Gozalo-Marcilla et al. 2013) but, to date, the effect on MAC of adding a dexmedetomidine CRI to a morphine infusion has not been studied.

Traditionally, the concept of the MAC is defined as the alveolar concentration of volatile anaesthetic agent at which 50% of the patients do not respond with purposeful movement to a supramaximal noxious stimulus (Merkel & Eger 1963). Differentiation between purposeful versus nonpurposeful movement is sometimes difficult and subjective. Derivatives of the traditional MAC such as the minimum end-tidal concentration of sevoflurane necessary to prevent movement (MAC_{NM}), have therefore

been described in the literature. From a clinical standpoint, MAC_{NM} may be more relevant than the traditional MAC (Seddighi et al. 2011, 2012).

The main objectives of this study were to determine and compare the MAC_{NM} values of sevoflurane in experimental ponies receiving a CRI of morphine alone or combined with dexmedetomidine.

Materials and methods

The experiment was approved by the Local Ethical Committee of the Faculty of Veterinary Medicine of the University of Ghent (2011/168).

Animals and instrumentation

Five healthy gelding ponies, aged 13 ± 3 years, weighing 294 ± 57 kg with body condition scores of four out of five (Carroll & Huntington 1988) were included in this trial.

Food, but not water, was withheld for twelve hours. Induction and maintenance of anaesthesia, fluid therapy and monitoring was performed as described by Gozalo-Marcilla et al. (2013). Briefly, anaesthesia was induced in the recovery box with sevoflurane (Sevorane, Abbott, Belgium) in oxygen via a nasotracheal tube. After induction, the ponies were positioned on a surgical table in right lateral recumbency. General anaesthesia was maintained with sevoflurane in oxygen/air [inspired oxygen fraction (FiO_2) of 55%]. The ponies were mechanically ventilated [intermittent positive pressure ventilation (IPPV) with tidal volume of 10 mL/kg and positive end-expiratory pressure (PEEP) of 0.49 kPa (5 cmH₂O)] to maintain the arterial partial pressure of carbon dioxide ($PaCO_2$) between 6.67-8.00 kPa (50-60 mmHg). Lactated Ringer's solution (Hemofiltratie BH 504, Dirinco, The Netherlands) was infused IV (3 mL/kg/hr) and a urinary catheter was placed.

Monitoring included electrocardiography, pulse oximetry, anaesthetic gas monitoring, invasive (transposed carotid artery) and non-invasive (right metacarpal artery) blood pressure (S/5 D-LCC15-03, Datex Ohmeda, OR, USA) and body temperature by a nasal probe. Arterial blood samples were withdrawn for blood gas analysis [pH, standard base excess and arterial partial pressures of oxygen (PaO_2) and $PaCO_2$ (ABL5, Radiometer, Denmark)]. A warm-water blanket was placed under the

ponies, bubble wrap was used to cover the ponies and the room temperature was maintained at 23°C.

According to our protocol, dobutamine would be infused to maintain the mean arterial pressure (MAP) over 65 mmHg in case MAP decreased below 60 mmHg and the FiO_2 would be increased if severe hypoxaemia was present (PaO_2 below 60 mmHg).

Experimental design

Each pony was anaesthetised twice for treatment M (morphine) or DM (dexmedetomidine plus morphine), in a randomized order with a three weeks washout period. The main anaesthetist (MGM) was unaware of the treatment.

Once on the table (T0), anaesthesia was maintained for sixty minutes (T60) with sevoflurane at an expired fraction ($F_{E'}\text{SEVO}$) of 2.5%. At T10, the ponies received an IV bolus of morphine (0.15 mg/kg) (Morphine.HCl, Sterop, Belgium), combined with either a bolus of dexmedetomidine (3.5 $\mu\text{g}/\text{kg}$) (Dexdomitor, Orion Corporation, Finland) (treatment DM) or an equivalent volume of saline (treatment M), over five minutes (T10-T15). The different boluses were administered slowly by hand. From T15 onwards, a morphine CRI was administered (0.1 mg/kg/hr), combined with dexmedetomidine (1.75 $\mu\text{g}/\text{kg}/\text{hr}$) (treatment DM), or saline, at the same volume and rate (treatment M) until the end of the anaesthesia.

MAC_{NM} determination

The MAC_{NM} of sevoflurane was determined applying constant current (CC) electrical stimuli to the skin of the lateral pastern region by means of an electrical stimulator (Grass S88, Grass medical instruments, MA, USA) equipped with a CC unit (Grass Constant Current Unit, Grass Technologies, RI, USA).

At T60, the first CC stimulus was applied as previously described by Gozalo-Marcilla et al. (2013). The response to electrical stimulation was assessed by the blinded anaesthetist who was allowed only to monitor reflex suppression, muscle tone and gross response to noxious stimulation. Positive reactions were considered gross purposeful movements, swallowing, generalized muscle tremors, movement of the head, limbs, ears or tail, or spontaneous movements without electrical stimulation. Nystagmus and physiological parameter modifications were considered negative responses. If the

reaction was negative, the $F_E \text{ SEVO}$ was decreased by 0.2%. Once this level was reached, it was maintained for twenty minutes before the next stimulation. This process was repeated until a positive response was obtained. If the first stimulus elicited a positive response, $F_E \text{ SEVO}$ was increased in 0.2% steps until a negative response was obtained. The MAC_{NM} was determined as the average of the lowest concentration preventing a positive response and the highest allowing a positive response. Triplicate MAC_{NM} estimations were obtained, corrected to standard atmospheric pressure and averaged in each pony. The correction to standard atmospheric pressure ($MAC \times P_B / 101.325 \text{ kPa}$; where P_B was the ambient barometric pressure at the moment of each MAC estimation) was performed as reported by Mama et al. (1999), and using the data available from the Royal Meteorological Institute (RMI) of Belgium, which monitors hourly the exact P_B at a location close to our institution.

Recording of cardiopulmonary variables and arterial blood gases

Cardiopulmonary values were recorded every ten minutes and arterial blood gas samples collected every twenty to thirty minutes. Cardiovascular data was recorded immediately before and arterial blood gases immediately after each electrical stimulation. For both cardiopulmonary variables and arterial blood gases, the mean of the 3 values recorded respectively before and after each MAC_{NM} estimation was calculated.

Recovery period

After the determination of the third MAC_{NM} value, CRIs and sevoflurane were discontinued and once breathing spontaneously, the ponies received dexmedetomidine (0.875 $\mu\text{g}/\text{kg}$, IV) and were transported to the recovery box. Oxygen was insufflated (8 L/min) and intranasal phenylephrine (Phenylephrine 10%, Théa Pharma, Belgium) was administered into both nostrils to reduce post anaesthetic upper airway obstruction, with the nasotracheal tube in place. After swallowing, the nasotracheal tube was removed. Recoveries were manually assisted and scored by the same blinded anaesthetist using a scale of 1-5 (Table 1). Furthermore, recovery times including times to extubation and times to regain sternal recumbency and standing position were recorded.

Table 1: Scoring system used to grade recovery after sevoflurane anaesthesia in five adult ponies.

Score	Description
1	One attempt to stand, no ataxia.
2	One to two attempts to stand, some ataxia.
3	More than two attempts to stand but quiet recovery.
4	More than two attempts to stand, excitation.
5	Severe excitation. Pony injured.

Statistical analysis

For the sevoflurane MAC_{NM} values, analysis of variance with treatment and period as fixed factors was used to detect differences between treatments ($\alpha = 0.05$). As correctness of the model could not be confirmed by analysis of the residuals (Kolmogorov-Smirnov test) a non-parametric test for paired samples (Wilcoxon signed-rank test) was performed. Analyses of variance were used to compare duration of anaesthetics and total amount of morphine administered.

Results

MAC_{NM} determination

Sevoflurane MAC_{NM} values (mean \pm SD) for treatments M and DM were $2.79 \pm 0.73\%$ and $0.89 \pm 0.22\%$ respectively, indicating a mean MAC_{NM} reduction of $67 \pm 11\%$ (ranging from 53 to 78%) as shown in Table 2.

The responses were clear in all the ponies after electric stimulation, except for ponies 1 and 3 during treatment M, in which maintenance of anaesthesia was more difficult, with frequent spontaneous movements and fighting against the ventilator.

Table 2: Individual mean MAC_{NM} values (%) for sevoflurane with a morphine infusion (treatment M) and for sevoflurane with morphine and dexmedetomidine infusions (treatment DM) in five adult ponies.

Pony	Treatment M	Treatment DM	% MAC _{NM} reduction
1	4.07	0.89	78
2	2.48	0.74	70
3	2.56	0.62	76
4	2.21	1.04	53
5	2.65	1.15	57
Mean \pm SD	2.79 ± 0.73	0.89 ± 0.22	67 ± 11

Values corrected to one atmosphere at sea level (760 mmHg).

Cardiopulmonary system

Heart rate and arterial blood pressures were maintained within acceptable limits. No dobutamine was required in any of the ponies receiving either treatment. During treatment M, PaO₂ in pony 1 decreased below 60 mm Hg (down to 48 mmHg at T150). In this case, the FiO₂ was increased to 100%, but no change in PaO₂ was observed (50 mmHg at the end of the anaesthetic, at T240). Moreover, moderate hypoxaemia (PaO₂ 60-80 mmHg) occurred in ponies 3 and 4 receiving treatment M. When receiving treatment DM moderate hypoxaemia was present in pony 1 while pony 4 showed a mild degree of hypoxaemia (80-90 mmHg). No significant differences in PaO₂ were observed between treatments. Cardiopulmonary data are shown in Table 3.

Table 3: Cardiopulmonary parameters, body temperature and duration of anaesthesia in five sevoflurane anaesthetized ponies (mean \pm SD) that received a morphine infusion (treatment M) or a morphine and dexmedetomidine infusion (treatment DM).

Variable	Unit	Treatment M	Treatment DM
Heart rate	beats/min	45 \pm 2	37 \pm 5
Systolic arterial pressure	mmHg	139 \pm 23	132 \pm 23
Mean arterial pressure	mmHg	118 \pm 20	106 \pm 17
Diastolic arterial pressure	mmHg	101 \pm 16	90 \pm 16
Arterial pH		7.42 \pm 0.03	7.42 \pm 0.02
Standard base excess	mmol/L	7 \pm 2	9 \pm 1
PaCO ₂	kPa	6.7 \pm 0.7	7.1 \pm 0.1
	mmHg	50 \pm 5	53 \pm 1
PaO ₂	kPa	12.4 \pm 7.2	16.5 \pm 7.6
	mmHg	93 \pm 54	124 \pm 57
Body temperature	°C	37.3 \pm 0.4	37.1 \pm 0.5
Duration of anaesthesia	min	168 \pm 56	333 \pm 35

Partial pressure of oxygen (PaO₂) and carbon dioxide (PaCO₂).

Values were obtained from three data collection periods before minimal alveolar concentration no movement (MAC_{NM}) determination (mean \pm SD).

Duration of anaesthesia, total amount of morphine administered, and recovery times and quality

Duration of anaesthesia (mean \pm SD) was shorter for treatment M than treatment DM (168 \pm 56 versus 333 \pm 35 min) (Table 3). The total amount (mean \pm SD) of morphine received per pony per anaesthesia was 0.43 \pm 0.09 and 0.71 \pm 0.06 mg/kg for treatments M and DM respectively ($p < 0.001$).

No significant differences were found in the recovery times (Table 4). All recoveries were scored as 1 (one attempt to stand, no ataxia) except for pony 1 after treatment M, where the recovery was scored as 2 (two attempts, slight ataxia) and signs of excitement and over-reactions to stimuli were observed until two hours after the end of the anaesthesia. After standing, pony 3, which had received treatment M (morphine only), walked in circles in the recovery box until two hours after anaesthesia.

Table 4: Time (mean \pm SD) to extubation, sternal and standing and recovery scores in five sevoflurane anaesthetized ponies that received a morphine infusion (treatment M) or a morphine and dexmedetomidine infusion (treatment DM).

Pony	Treatment	Extubation time (minutes)	Time to sternal (minutes)	Time to stand (minutes)	Recovery scores
1	M	12	13	16	2
2	M	7	20	21	1
3	M	7	14	17	1
4	M	9	11	14	1
5	M	8	8	9	1
Mean \pm SD	M	9 \pm 2	13 \pm 4	15 \pm 4	
1	DM	9	9	14	1
2	DM	5	16	16	1
3	DM	10	10	21	1
4	DM	4	10	11	1
5	DM	1	2	7	1
Mean \pm SD	DM	6 \pm 4	9 \pm 5	14 \pm 5	

Discussion

The results of this study show that co-administration of dexmedetomidine and morphine CRIs significantly reduces the MAC_{NM} of sevoflurane compared with a CRI of morphine alone at the reported doses (mean \pm SD reduction of $67 \pm 11\%$).

When determining the MAC_{NM} values in the present study, not all the responses were clear movements after electrical stimulation, and in ponies 1 and 3 during treatment M, maintenance of anaesthesia was more difficult, with the occurrence of generalized muscle tremors and fighting against the ventilator. These differences may be explained by a combined analgesic, excitatory and locomotor stimulant effect of morphine in horses (Kamerling et al. 1989), with possible inter-individual differences. Furthermore, the findings of two research papers studying the effects of opioids in horses (Pascoe et al. 1993; Steffey et al. 2003) suggested that the MAC technique may not be able to differentiate between arousal of the central nervous system (CNS) or stimulation and suppression of response to noxious stimulation (i.e. analgesia). Co-administration of dexmedetomidine seems to avoid these reactions, most probably due to its sedative properties.

One of the limitations of this study is that it was performed under experimental conditions in healthy animals. It cannot be excluded that the effects of morphine might be different in clinical patients undergoing surgery with pre-existing pain and caution should be taken when interpreting these results. However, it is generally accepted that the results of MAC studies are useful in predicting the required end-tidal anaesthetic agent concentration that will result in a surgical depth of anaesthesia in patients (Quasha et al. 1980). Another important limitation is the absence of a control (saline) group. Ideally, four different treatments (treatments M and DM, as well as treatments S and D) would have been included, all administered in a randomized order, to describe the effects of a morphine CRI on the MAC_{NM} , either when administered alone or combined with dexmedetomidine. However, since MAC_{NM} with treatments S and D had already been determined in a previous experiment in the same ponies, under identical circumstances and less than five months before the present experiment (Gozalo-Marcilla et al. 2013), it was considered unethical to repeat treatments S and D. Although a statistical comparison was therefore only made between treatments M and DM, in the authors' opinion it still remains interesting to compare the present results to those of our previous report.

The MAC_{NM} value (mean \pm SD) for treatment M obtained in this study was $2.79 \pm 0.73\%$, which is somewhat higher than the value obtained under the same conditions when receiving saline (2.42 ± 0.55) (Gozalo-Marcilla et al. 2013). Individually, the MAC of four out of five ponies was higher when receiving morphine compared to saline and did not change in the remaining pony (pony 4). Administration of boluses at low and high doses of morphine (0.25 mg/kg and 2.0 mg/kg, respectively) increased, decreased or did not influence the MAC of isoflurane in horses, which does not support the routine use of morphine as an anaesthetic adjuvant in horses (Steffey et al. 2003). The influence of morphine on MAC thus appears to be less consistent in the equine than in other species (Steffey et al. 1994), and Steffey et al. (2003) were unable to identify any specific characteristic that would allow them to predict each horse's individual MAC response to morphine administration.

Compared to the use of morphine alone, the co-administration of dexmedetomidine and morphine CRIs produced a significant reduction of the sevoflurane MAC_{NM} by $67 \pm 11\%$. A reduction was found in all the ponies, which

ranged between 53 and 78%. Moreover, during treatment DM the MAC_{NM} was even lower (0.89 ± 0.22 %) than during treatment D in our previous report (1.07 ± 0.21 %). Therefore, it appears likely that dexmedetomidine reduces the excitatory CNS effects of a morphine CRI in anaesthetized horses, but perhaps preserves, or even enhances, morphine's analgesic effects. In standing horses, the administration of α_2 -agonists and opioids produces synergistic analgesic effects, with reduced adverse side effects (Clarke & Paton 1988; Solano et al. 2009). Intraoperatively, the addition of dexmedetomidine to morphine in human patients resulted in superior analgesia, significant morphine sparing and less morphine-induced nausea, without additional sedation and untoward haemodynamic changes (Lin et al. 2009).

With regard to the recovery times, no significant differences were found between treatments. Possibly, administration of dexmedetomidine ($0.875 \mu\text{g}/\text{kg}$, IV) prior to recovery in both treatment groups masked potential differences. However, the times to sternal recumbency and to standing in both groups were shorter when compared to the values of our previous study with saline and dexmedetomidine CRIs (Gozalo-Marcilla et al. 2013). This may be related to the use of morphine, since, Clark et al. (2008) reported shorter times from the first recovery movement to the time at standing in horses receiving a bolus and an infusion of morphine at the dose and rates reported here. Surprisingly, the addition of dexmedetomidine to morphine did not increase but shortened the recovery times in all but one pony, despite the considerably longer duration of anaesthesia. Possibly, this finding can be attributed to the effects of morphine accumulation. Although the morphine plasma concentration was not determined in the present study, the duration of general anaesthetics, and consequently the cumulative doses of morphine, were significantly higher in ponies during treatment DM. It may be hypothesized that the sedative effects of dexmedetomidine were overcome by the larger accumulated dose of morphine, leading to CNS stimulation, accelerating return from anaesthesia with recovery times becoming shorter.

In addition to the difficulties for maintenance of general anaesthesia, pony 1 required two attempts to stand after treatment M and showed excitement and ataxia with over-reaction to stimuli for up to two hours after anaesthesia. Although pony 3 after treatment M needed only one attempt to stand with no ataxia (score 1), he later showed clear signs of box-walking for up to two hours after treatment M. The simultaneous

infusion of dexmedetomidine during treatment DM seemed to avoid these reactions, as all the ponies recovered well (all score 1) and did not show any sign of excitation or box-walking during the recovery period. In the study reported by Steffey et al. (2003), IV morphine at 2.0 mg/kg produced dangerous recoveries but not at low doses. Good recoveries were described in horses receiving lower doses (0.1-0.2 mg/kg, IV) (Mircica et al. 2003; Love et al. 2006) and after a bolus and a CRI at the dose and rates reported here (Clark et al. 2008). No box-walking was observed during the post-operative periods after saline or dexmedetomidine infusions (Gozalo-Marcilla et al. 2013) and after single morphine boluses (Mircica et al. 2003; Love et al. 2006) or infusions (Clark et al. 2008).

Although comparison of cardiopulmonary function between both CRIs, including cardiac output, mixed venous blood gases, etc., would have been interesting, this was not the primary aim of the study and this comparison would have been difficult due to the large difference in duration of anaesthesia. Heart rate and arterial blood pressures were maintained within clinically acceptable limits (Table 3). However, despite IPPV and PEEP, severe hypoxaemia ($\text{PaO}_2 = 48 \text{ mmHg}$) was present in pony 1 receiving treatment M. Consequently, 100% oxygen was administered, but PaO_2 remained low. Presence of severe hypoxaemia could have an influence on MAC_{NM} values, but this only has been reported with PaO_2 values lower than 40 mmHg (Steffey & Mama 2007). Moreover, although mean ($\pm \text{SD}$) values of PaO_2 were lower in ponies receiving morphine, no statistically significant difference was found between treatments. In previous studies, horses receiving morphine had lower PaO_2 values (Steffey et al. 2003; Love et al. 2006), mainly related to hypoventilation, although no differences were found when morphine was administered as a CRI (Clark et al. 2005). Furthermore, ventilation/perfusion mismatch related to the high body condition score of the ponies (Moens 1989) may have been a contributing factor, exacerbating hypoxaemia in the present study, as all five ponies were classified as fat according to the body condition scoring reported by Carroll & Huntington (1988).

In conclusion, co-administration of dexmedetomidine and morphine CRIs significantly reduced the MAC_{NM} of sevoflurane compared with a CRI of morphine alone. Furthermore, the combination of both drugs could be used in equine anaesthesia, thus reducing the needs of inhalant agents and possibly enhancing analgesia. Potential

CNS stimulation should be considered when using a morphine CRI alone, since generalized and uncontrolled spontaneous movements in the intraoperative period and excitation, overreaction to stimuli and box-walking during the recovery period could be present after prolonged infusions.

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Chapter 5

Comparison of the influence of two different constant rate infusions (dexmedetomidine versus morphine) on anaesthetic requirements, cardiopulmonary function and recovery quality in isoflurane anaesthetized horses

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Abstract

Twenty adult healthy horses undergoing elective surgery were involved in this prospective, blinded, clinical study. Horses were randomly allocated to receive a constant rate infusion (CRI) of morphine or dexmedetomidine. After induction, anaesthesia was maintained with isoflurane in oxygen/air and mechanical ventilation applied. The end-tidal isoflurane concentration ($F_E \text{ ISO}$) was initially set at 0.9% and adjusted by the anaesthetist, to maintain a light surgical plane of anaesthesia, according to an objective flow-chart. Cardiopulmonary function was well maintained with both treatments. Less ketamine was required, $F_E \text{ ISO}$ was lower after one hour and more time was spent in an 'ideal' plane of anaesthesia in horses receiving dexmedetomidine, with better recoveries. One horse receiving morphine developed post-operative colic and pulmonary oedema and two showed box-walking behaviour. This study showed that a dexmedetomidine CRI produced a more stable anaesthetic depth, reduced isoflurane requirements and better recoveries, without post-operative complications compared with a morphine CRI.

Introduction

The use of intravenous (IV) constant-rate infusions (CRIs) of α_2 -agonists has been widely studied in horses (Bettschart-Wolfensberger et al. 2001; Devisscher et al. 2010; Schauvliege et al. 2011; Pöppel et al. 2012). Dexmedetomidine, the dextro-rotary and active enantiomer of the racemic mixture medetomidine, is currently the most potent and selective α_2 -agonist which is commercially available for use in humans and small animals. Experimental studies in isoflurane anaesthetized ponies showed that dexmedetomidine at two different rates maintained cardiovascular function within clinically acceptable limits and produced the typical cardiopulmonary effects of α_2 -agonists, while the arterial partial pressure of oxygen (PaO₂) tended to be low (Marcilla et al. 2010). In a blinded clinical study involving forty healthy isoflurane anaesthetized horses undergoing elective surgery, dexmedetomidine (3.5 µg/kg followed by a CRI 1.75 µg/kg/hr) produced no clinically relevant effects on the cardiovascular system compared with a placebo group. Although PaO₂ was significantly lower in horses receiving dexmedetomidine, oxygen delivery indexed to weight (DO₂I) was comparable between groups (Marcilla et al. 2012). Moreover, a dexmedetomidine infusion improved the quality of the recovery. Although no reduction in expiratory fraction of isoflurane (F_E ISO) was demonstrated, a minimal alveolar concentration (MAC) study performed in six ponies showed that the same protocol reduced the MAC of sevoflurane by 53 ± 15% (mean ± SD) (Gozalo-Marcilla et al. 2013a). As a dexmedetomidine CRI causes minor cardiopulmonary effects, improves recovery quality and reduces the MAC of inhalant agents, the proposed protocol could be useful for clinical equine balanced anaesthesia (Bettschart-Wolfensberger & Larenza 2007).

The use of systemically administered opioids in horses to provide analgesia remains controversial (Bennett & Steffey 2002; Clutton 2010). Some authors reported that morphine administered IV can induce dangerous behaviour in conscious (Combie et al. 1979) and anaesthetized horses (Steffey et al. 2003), causing post-operative colic (Roger et al. 1985) and respiratory depression (Steffey et al. 2003). In contrast, other investigators reported minimal haemodynamic and ventilatory changes (Mircica et al. 2003; Clark et al. 2005) without an increased incidence of post-operative colic (Mircica et al. 2003). Even more, improved recovery qualities were observed (Mircica et al. 2003; Love et al. 2006; Clark et al. 2008) after boluses and CRIs of morphine in horses

undergoing elective surgical procedures. With regard to anaesthetic sparing effects, the IV administration of boluses of morphine at two different doses (Steffey et al. 2003) failed to reduce the MAC of isoflurane. Moreover, the minimum end-tidal concentration to prevent movement (MAC_{NM}) value of sevoflurane obtained in experimental ponies receiving a morphine CRI (0.15 mg/kg/hr after loading dose 0.1 mg/kg) was higher than the one obtained under the same conditions when receiving saline (Gozalo-Marcilla et al. 2013b). Both studies were performed in healthy, pain-free horses under experimental conditions, where the applied electrical stimulation was qualitatively different from classic surgical nociception (Clutton 2010). In contrast, clinical equine patients under a morphine CRI tended to receive fewer and lower doses of additional anaesthetic drugs, although this was not of statistical significance (Clark et al. 2005).

The aim of the present study was to evaluate and compare the cardiopulmonary function and recovery quality of clinically healthy horses undergoing elective surgery either receiving a morphine or a dexmedetomidine CRI at the doses reported by Clark et al. (2005) and Marcilla et al. (2012) respectively. Additionally, the hypothesis that maintenance of stable anaesthetic depth in horses receiving a CRI of dexmedetomidine would be easier compared with horses receiving a morphine CRI, requiring a lower F_E ISO and less additional ketamine was investigated.

Materials and methods

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine of Ghent University (2010/177).

Animals and instrumentation

After obtaining written owner consent, data from twenty adult healthy horses [American Society of Anesthesiologists (ASA) category I or II] undergoing elective surgery (soft tissue and orthopaedic procedures) lasting more than one hour were included in this clinical study. The horses were aged between ten months and fifteen years old, weighing 466 ± 117 kg (mean \pm SD). Head or neck surgeries were excluded due to the difficulty of assessment of the clinical parameters related to anaesthetic depth. Respecting the current EU laws of medication in animals, horses classified as ‘food

producing animals' were excluded as dexmedetomidine is not licensed for use in the horse.

The horses were randomly allocated to receive treatment M (morphine) or treatment D (dexmedetomidine). Food, but not water, was withheld for twelve hours before anaesthesia and pre-anaesthetic examinations were performed the evening before the surgical procedure. All the anaesthetic procedures were performed by the same anaesthetist (MGM), who was unaware of the treatment. All medication syringes were prepared by one of the co-authors. Sedation scores, depth of anaesthesia and recovery scores were evaluated by the same blinded anaesthetist.

The horses in treatment M received for sedation dexmedetomidine (3.5 µg/kg, IV) (Dexdomitor, Orion Corporation, Finland) plus morphine (0.15 mg/kg, IV) (Morphine.HCl, Sterop, Belgium) and those in treatment D received dexmedetomidine (3.5 µg/kg, IV) plus a bolus of saline of equivalent volume as morphine in treatment M. Depth of sedation was scored as 0 (no sedation), 1 (slight sedation), 2 (good sedation) or 3 (deep sedation). After sedation, a 12-gauge x 80 mm catheter (Intraflon 2, Ecoen, France) was placed in the jugular vein. If required, additional doses of dexmedetomidine (1/4 to 1/2 of the initial dose) were given to achieve an adequate level of sedation prior to anaesthetic induction. Anaesthesia was induced seven to ten minutes after sedation, with midazolam (0.06 mg/kg) (Dormicum, Roche, Belgium) and ketamine (2.2 mg/kg) (Anesketin, Eurovet, Belgium) IV mixed in the same syringe.

After orotracheal intubation (24-30 mm OD tracheal tube, Willy Rusch AG, Germany) the horses were hoisted on a surgical table covered with soft foam rubber pillows (twenty cms) and positioned according to the planned surgical procedure. The endotracheal tube was connected to a large animal anaesthetic unit (Matrx Medical Inc., NY, USA mounted on a Sulla 909V; Dräger, Germany) with an out-of-circuit vaporizer (Drägerwerk AG, Germany) and a large animal ventilator (Smith respirator LA 2100, model 2002, Veterinary Technics/BDO-Medipass, The Netherlands). Connection to the anaesthetic circuit was considered as the beginning of anaesthesia (T0). Anaesthesia was maintained with isoflurane (Isoflo, Abbott Laboratories Ltd, UK) in a mixture of oxygen (O₂) and air to maintain the inspired O₂ fraction (FiO₂) between 55 and 60%. All the horses were mechanically ventilated immediately after positioning on the table. Intermittent positive pressure ventilation (IPPV) was applied, using an assisted-

controlled respiration mode, with a tidal volume of 10 mL/kg, peak inspiratory pressure close to 1.96 kPa (20 cm H₂O), inspiratory time of around 2.2 seconds and respiratory rate near to 8 breaths/min. All parameters were adapted to maintain arterial partial pressure of carbon dioxide (PaCO₂) between 4.66 and 6.00 kPa (35-45 mmHg).

Inspiratory and expiratory CO₂, O₂ and isoflurane concentrations were measured using a calibrated, methane-insensitive, multiparameter monitoring device (S/5, D-LCC15-03, Datex Ohmeda, OR, USA). This monitor was also used to record the electrocardiogram (base-apex lead), systolic (SAP), diastolic (DAP) and mean arterial pressures (MAP), peripheral arterial saturation by pulse oximetry (probe on the tongue) and body temperature by a nasal probe.

Catheterization of the facial artery was performed in all horses (22-gauge Vasocan Braunüle Luer Lock; B. Braun Melsungen AG, Germany) to obtain arterial blood for analysis, for withdrawal of blood for lithium dilution cardiac output measurements and for continuous invasive measurement of arterial blood pressures. The pressure monitoring system was zeroed at the level of the right atrium.

Cardiac output (\dot{Q}_t) was determined using the lithium dilution technique (LiDCOplus Haemodynamic Monitor, LiDCO Ltd., UK). A bolus of lithium chloride (4.5 μ mol/kg) was injected through the jugular catheter, while arterial blood for detection of lithium chloride by the LiDCO sensor (CM10 LiDCO sensor, LiDCO Ltd., UK) was withdrawn from the facial artery by the LiDCO Flow Regulator (CM 33 LiDCO flow regulator, LiDCO Ltd., UK). Plasma sodium values were determined (AVL 9180 Electrolyte Analyzer, AVL Scientific Corporation, GA, USA) on a blood sample withdrawn from the right jugular vein before sedation and were entered into the LiDCOplus monitor to allow correct LiDCO measurements. Haemoglobin (Hb) concentration was estimated for each measurement from the packed cell volume (PCV) [Hb (g/dL) = 34 x PCV (L/L) (Linton et al. 2000)].

Intraoperatively, all the horses received flunixin meglumine (1.1 mg/kg, IV) (Endoflurin 50, Ecuphar, Belgium) and intramuscular procaine benzylpenicillin (15000 IU/kg) (Pen-30, V.M.D., Belgium).

Experimental design

The vaporizer was adjusted to obtain an $F_E \text{ ISO}$ of 0.9% during the first ten minutes of general anaesthesia. Horses allocated to treatments M and D received at T0 a CRI of morphine (0.1 mg/kg/hr) and dexmedetomidine (1.75 $\mu\text{g/kg/hr}$) respectively. Constant rate infusions were administered by a syringe driver (Ohmeda 9000, BOC Health Care, UK) until the end of anaesthesia. Lactated Ringer's solution (Hamofiltrație, Dirinco, The Netherlands) was administered IV (10 mL/kg/hr). A urinary catheter was placed in all the horses.

Instrumentation was completed by T10. Values for inspiratory CO_2 , O_2 , isoflurane, heart rate (HR), SAP, MAP, DAP and body temperature were recorded every five minutes. Cardiac output was measured and arterial blood samples collected at fifteen minutes intervals (ABL5, Radiometer, Denmark).

Cardiac output indexed to bodyweight (CI), stroke volume (SV), stroke volume indexed to bodyweight (SVI), systemic vascular resistance (SVR), arterial oxygen content (CaO_2), oxygen delivery (DO_2) and DO_2I were calculated using standard formulae (Schauvliege et al. 2008).

Maintenance of anaesthesia

Dobutamine (Dobutamine EG, NV Eurogenics, Belgium) was infused to maintain MAP above 70 mmHg, starting at a rate of 0.5 $\mu\text{g/kg/min}$ and adjusted as required. The $F_E \text{ ISO}$ was adjusted to maintain a light surgical plane of anaesthesia by the blinded anaesthetist, according to a scoring flow-chart adapted from Enderle et al. (2008) (Figure 1) in order to assess the depth of anaesthesia more objectively. After T10 ($F_E \text{ ISO} = 0.9\%$), a scoring evaluation was performed every five minutes. For each scoring, the reference value was the previous score. The flow-chart ranges from scores -2 (deep anaesthesia) to 4 (very light anaesthesia, limb movement present), where score 0 was considered as the 'ideal' surgical plane of anaesthesia. The vaporizer setting was adjusted to provide a higher or lower $F_E \text{ ISO}$ according to occurrence of movement (scores 3 and 4), nystagmus (score 2), presence of palpebral reflex (scores -2 to 1) and absence of palpebral reflex (score -2). If a palpebral reflex was present, with no movement neither nystagmus (scores -2 to 1), scoring was mainly based on MAP values and the administration (or no administration) of dobutamine. If MAP was lower than 70

mmHg, the F_E ISO was reduced by 0.1% (score -1). If MAP was higher than 70 mmHg, the decision was based on changes in MAP compared to the previous value, while taking possible changes in the administration rate of dobutamine into account: if MAP was reduced by more than 15% compared with the previous scoring, a score of -2 was given and the F_E ISO was reduced by 0.2%. If MAP was reduced by 15% or less and a dobutamine infusion had been started or its rate increased during the last 5 minutes, a score -1 was given and the F_E ISO was reduced by 0.1%; if the administration rate of dobutamine had not changed in the last 5 minutes, the anaesthetic depth was considered ideal (score 0) and the F_E ISO was maintained constant. A score 0 was also given when MAP increased by less than 20% or when it increased by more than 20% when the dobutamine administration rate had been increased in the last 5 minutes. Increases in MAP by more than 20% without changes in the out changes in the dobutamine administration rate indicated too light anaesthesia (score 1) and F_E ISO was increased by 0.1%. If movement or nystagmus were present (scores 2 to 4), ketamine was administered IV as indicated in the flow-chart. Ketamine doses and dobutamine rates were calculated for each horse and corrected according to the individual body weight and anaesthesia duration. Percentages of the time of anaesthesia spent in each score were calculated for every horse from each treatment.

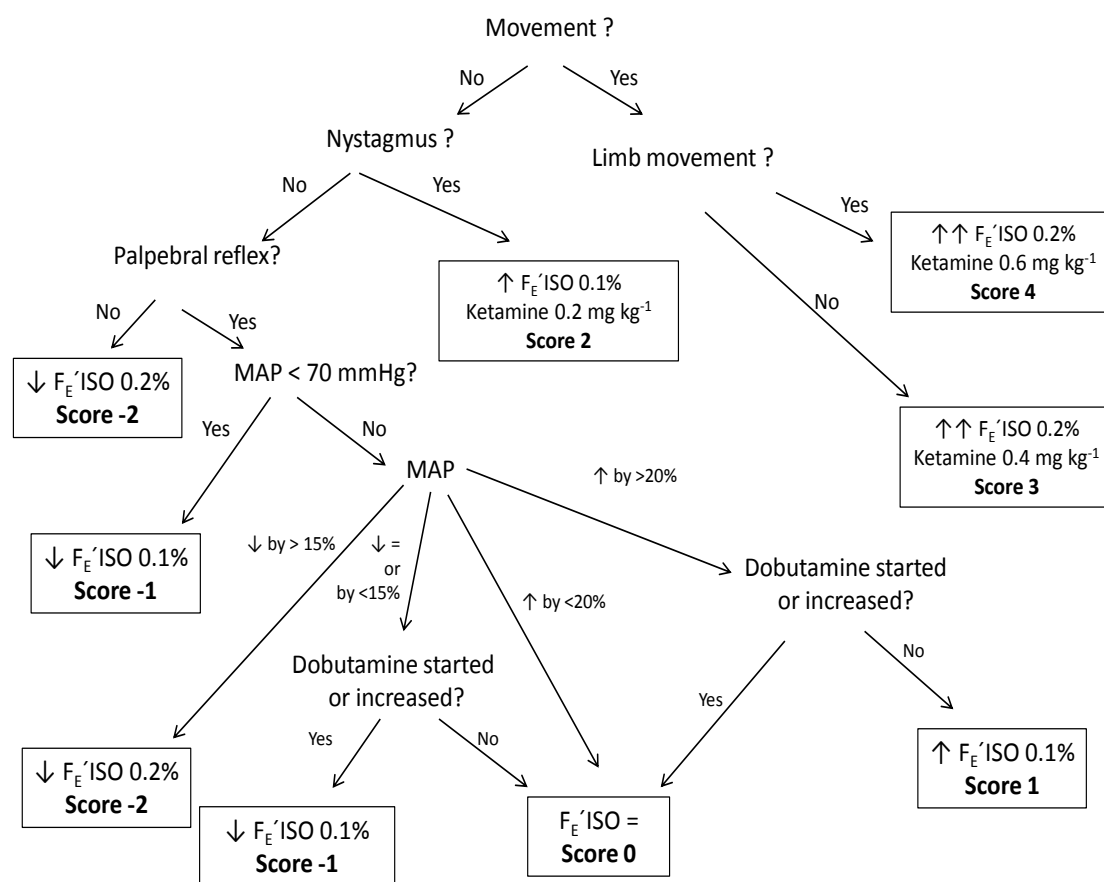


Figure 1: Flow-chart representing the scoring system used to adjust anaesthetic requirement in order to maintain a light surgical level of anaesthesia in twenty isoflurane anaesthetized horses undergoing elective surgery. Different questions are answered to obtain the score and the modification of the expiratory fraction of isoflurane ($F_E'ISO$) every five minutes, with the value obtained five minutes earlier being the reference value, and MAP the mean arterial pressure. Adapted from Enderle et al. (2008).

End of anaesthesia and recovery phase

At the end of the surgery, the CRIs and mechanical ventilation were stopped. Once breathing spontaneously, dexmedetomidine ($0.875 \mu\text{g}/\text{kg}$, IV) was administered in all horses which were transported to a padded recovery box and allowed to recover without assistance. Oxygen was administered initially through the endotracheal tube and nasally after extubation. The endotracheal tube was removed once the horses were able to

swallow. The rest of the recovery was monitored from outside the recovery box using an infrared camera system. Recovery times (extubation time, time to sternal recumbency and time to stand) were recorded and recovery qualities scored on a scale of 1-6 (Table 1) by the same blinded anaesthetist.

Table 1: Scoring system used to grade recoveries.

Score	Description
1	One attempt to stand, no ataxia.
2	One to two attempts to stand, some ataxia.
3	More than two attempts to stand, calm recovery.
4	More than two attempts to stand, not calm recovery.
5	Several attempts to stand, excitation.
6	Very bad recovery.

Statistical analysis

Data were tested for normality of distribution (Kolmogorov-Smirnov test). The age and weight of the horses, duration of anaesthesia, total doses of additional ketamine and dobutamine, sedation, depth of anaesthesia and recovery scores and recovery times were compared between treatments using a Wilcoxon rank sum test.

The duration of anaesthesia did not exceed sixty minutes in some horses. Consequently, only the data until T60 were analyzed. Differences between treatments were analyzed, overall and at T60 using a mixed model analysis of variance with horse as random effect and treatment, time and their interaction as categorical fixed effects. For all analyses the significance level was set at 5%.

Results

Age (5 ± 5 and 7 ± 5 years old, for treatment M and D respectively) and weight (treatment M 440 ± 125 kg and treatment D 491 ± 110 kg) did not differ statistically between groups. Nine horses were placed in dorsal recumbency and one in lateral recumbency in treatment M. Five horses receiving treatment D were placed in dorsal recumbency and the remaining five in lateral recumbency. Details of the surgical interventions are represented in Table 2.

Table 2: Types of surgeries performed in horses receiving treatment M (n = 10) and treatment D (n = 10). Horses allocated in treatment M received a bolus of dexmedetomidine (3.5 µg/kg) plus morphine (0.15 mg/kg) IV followed by a CRI of morphine (0.1 mg/kg/hr). Horses allocated in treatment D received a bolus of dexmedetomidine (3.5 µg/kg) plus an equivalent volume of saline instead of morphine IV followed by a CRI of dexmedetomidine (1.75 µg/kg/hr).

Type of surgery	Treatment M	Treatment D
Arthroscopy	4	6
Cryptorchid	2	0
Umbilical hernia	2	0
Tenoscopy	1	0
Sarcoid excision	1	0
Street nail	0	2
Castration	0	1
Sarcoid cryosurgery	0	1

Level of sedation and sedation scores

Sedation scores were significantly lower (less sedation) in treatment M compared with treatment D ($p = 0.042$). Eight horses (six receiving treatment M and two treatment D) required an additional dose of dexmedetomidine to assure an acceptable level of sedation.

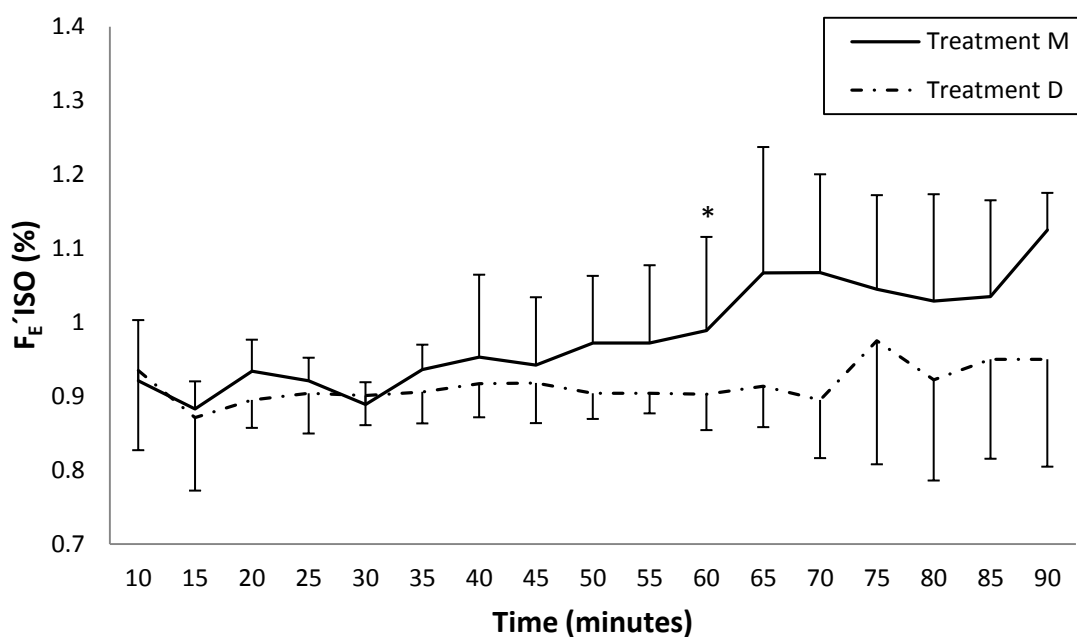
Isoflurane concentrations (Figure 2), anaesthetic depth scores (Figure 3) and requirements of ketamine and dobutamine

Although there was a significant influence of time on the $F_E \dot{V}_{ISO}$ ($p = 0.009$), no significant overall differences in $F_E \dot{V}_{ISO}$ were found between treatments. However, at T60, $F_E \dot{V}_{ISO}$ values were significantly ($p = 0.012$) lower in horses receiving treatment D compared to treatment M. Because the difference was gradually increasing over time, an additional statistical comparison was performed at T90 for the seven horses that were anaesthetized for ninety minutes or more (four treatment M versus three treatment D). At this time point, $F_E \dot{V}_{ISO}$ values tended to be lower in horses receiving treatment D, but the difference was not significant ($p = 0.069$).

Horses receiving treatment D spent significantly more time (in percentage) at score 0 compared to horses of treatment M (97.4% versus 79.7% respectively) ($p < 0.001$). Moreover, horses receiving treatment M spent significantly more time at score -1 than those receiving treatment D (7% versus 1.7% respectively) ($p = 0.043$).

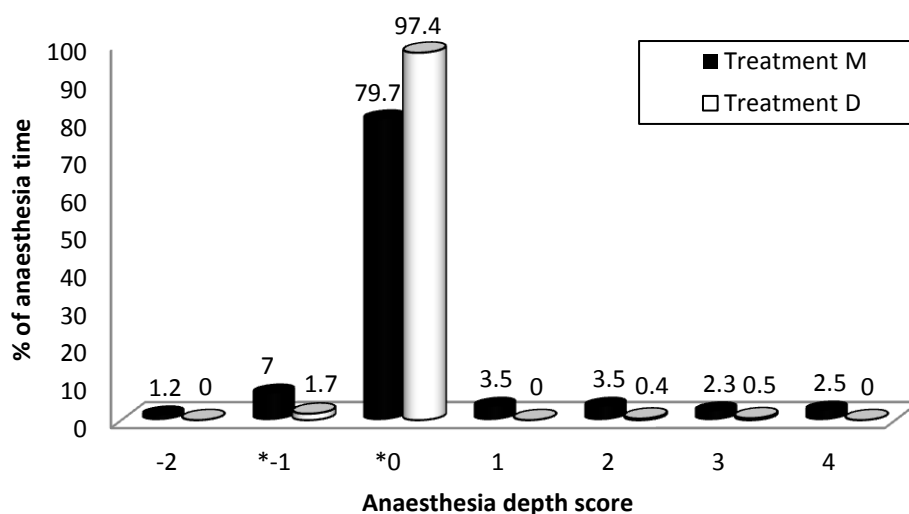
Nine horses receiving treatment M required additional doses of ketamine, whereas only two in treatment D received ketamine. The mean amount of ketamine administered (in $\mu\text{g}/\text{kg}/\text{min}$) was significantly higher in treatment M (8.71 ± 5.72) (mean \pm SD) versus treatment D (0.71 ± 1.58) ($p = 0.001$).

Six horses receiving treatment M and three horses receiving treatment D required dobutamine. The mean dose of dobutamine administered in horses receiving treatment M and D was 0.04 ± 0.04 and 0.01 ± 0.02 $\mu\text{g}/\text{kg}/\text{min}$ (mean \pm SD) respectively. This difference was not statistically significant ($p = 0.116$).



* Significant differences between treatments at T60 ($p < 0.05$). Changes over time were present ($p < 0.05$). Statistical analysis was not performed after T60.

Figure 2: End-tidal isoflurane concentration (F_E'ISO) (in percentage) (mean ± SD) in twenty horses anaesthetized with a standard isoflurane protocol for elective surgery. Horses in treatment M (n = 10) received a CRI of morphine (0.1 mg/kg/hr) and horses in treatment D (n = 10) received dexmedetomidine at 1.75 µg/kg/hr.



*Significant differences between treatments ($p < 0.05$).

Figure 3: Distribution of the anaesthetic time (in percentage) (mean values) spent at the different anaesthetic depth scores in twenty horses anaesthetized with a standard isoflurane protocol for elective surgery. Horses in treatment M (n = 10) received a CRI of morphine (0.1 mg/kg/hr) and horses in treatment D (n = 10) received dexmedetomidine at 1.75 μ g/kg/hr.

Cardiopulmonary system (Tables 3 & 4)

Overall, MAP ($p = 0.031$) and DAP ($p = 0.025$) were significantly higher whereas CI and SVI were significantly lower ($p = 0.009$ and $p = 0.028$, respectively) in horses receiving treatment D.

Statistical analysis also demonstrated gradual decreases over time of SAP, MAP, DAP, SVR and CaO_2 ($p < 0.0001$) in both treatments.

Table 3: Cardiovascular parameters (mean \pm SD) in twenty anaesthetized horses undergoing elective surgery using two different protocols.

Value	Differences	Unit	Treatment	T15	T30	T45	T60
HR		beats/min	M	34 \pm 5	34 \pm 4	34 \pm 5	34 \pm 4
			D	34 \pm 5	32 \pm 3	33 \pm 5	34 \pm 5
SAP	#	mmHg	M	122 \pm 12	103 \pm 9	102 \pm 13	105 \pm 21
			D	126 \pm 14	118 \pm 12	115 \pm 13	114 \pm 16
MAP	*, #	mmHg	M	99 \pm 9	80 \pm 9	79 \pm 13	82 \pm 20
			D	104 \pm 15	97 \pm 14	92 \pm 15	93 \pm 17
DAP	*, #	mmHg	M	85 \pm 9	65 \pm 9	66 \pm 15	68 \pm 20
			D	89 \pm 14	82 \pm 14	79 \pm 15	79 \pm 14
SVR	#	dyne/sec/cm ⁵	M	334 \pm 79	241 \pm 66	251 \pm 79	275 \pm 109
			D	394 \pm 159	344 \pm 105	313 \pm 117	294 \pm 102
CI	*	mL/kg/min	M	53.9 \pm 9.8	60.7 \pm 9.4	56.8 \pm 11.0	54.7 \pm 10.2
			D	45.0 \pm 9.3	45.8 \pm 9.3	48.0 \pm 4.9	48.5 \pm 6.1
SVI	*	mL/kg	M	1.6 \pm 0.2	1.8 \pm 0.3	1.7 \pm 0.3	1.6 \pm 0.3
			D	1.4 \pm 0.3	1.4 \pm 0.2	1.5 \pm 0.2	1.5 \pm 0.3

Heart rate (HR), systolic (SAP), mean (MAP) and diastolic arterial pressures (DAP), systemic vascular resistance (SVR), cardiac output indexed to weight (CI) and stroke volume indexed to weight (SVI) in twenty horses undergoing elective surgery. Horses receiving treatment M were infused a CRI of morphine (0.1 mg/kg/hr) and horses receiving treatment D a CRI of dexmedetomidine (1.75 μ g/kg/hr).

*Overall significant differences between treatments ($p < 0.05$). # Changes over time ($p < 0.05$).

Chapter 5

Table 4: Other cardiopulmonary and systemic parameters in twenty anaesthetized horses for elective surgery (mean±SD).

Values	Differences	Unit	Treatment	T15	T30	T45	T60
Body t ^a	#	°C	M	37 ± 0.6	37 ± 0.4	36.9 ± 0.4	36.8 ± 0.4
			D	37 ± 0.5	37 ± 0.5	36.9 ± 0.4	36.9 ± 0.4
Arterial pH			M	7.42 ± 0.05	7.42 ± 0.05	7.42 ± 0.06	7.41 ± 0.06
			D	7.46 ± 0.11	7.43 ± 0.03	7.43 ± 0.03	7.43 ± 0.04
Arterial PCO ₂		mmHg	M	45 ± 6	46 ± 7	47 ± 8	49 ± 9
			D	44 ± 6	45 ± 4	45 ± 4	47 ± 4
Arterial PCO ₂		kPa	M	6 ± 0.9	6 ± 0.9	6.2 ± 1.1	6.5 ± 1.2
			D	5.9 ± 0.8	6 ± 0.5	6 ± 0.5	6.2 ± 0.6
Arterial PO ₂		mmHg	M	201 ± 78	193 ± 69	185 ± 63	163 ± 53
			D	180 ± 79	171 ± 79	177 ± 76	177 ± 67
Arterial PO ₂		kPa	M	26.8 ± 10.4	25.7 ± 9.2	24.6 ± 8.4	21.8 ± 7
			D	24 ± 10.5	22.8 ± 10.5	23.9 ± 10.1	23.6 ± 8.9
CaO ₂	#	mL/L	M	143 ± 15	136 ± 16	131 ± 14	128 ± 16
			D	141 ± 15	137 ± 17	133 ± 15	129 ± 12
DO ₂ I		mL/kg/min	M	7.7 ± 1.8	8.3 ± 1.6	7.5 ± 1.8	7.1 ± 1.9
			D	6.3 ± 1.3	6.3 ± 1.7	6.4 ± 1	6.2 ± 1.1
PCV	#	%	M	29 ± 3	28 ± 3	27 ± 3	26 ± 3
			D	29 ± 3	28 ± 3	27 ± 3	27 ± 2

Body temperature (body t^a), arterial blood gas results, arterial oxygen content (CaO₂), oxygen delivery indexed to weight (DO₂I), and packed cell volume (PCV) in twenty anaesthetized horses undergoing elective surgery. Horses receiving treatment M were infused a CRI of morphine (0.1 mg/kg/hr) and horses receiving treatment D a CRI of dexmedetomidine (1.75µg/kg/hr). # Changes over time (p < 0.05).

Recovery scores (Table 5) and times

Recovery scores were significantly better in horses receiving treatment D ($p = 0.015$), with less attempts to stand ($p = 0.015$). No differences in recovery times were found between treatments.

Table 5: Recovery scores in twenty horses anaesthetized for elective surgery. Horses receiving treatment M (n = 10) were infused a CRI of morphine (0.1 mg/kg/hr) and horses in group D (n = 10) a CRI of dexmedetomidine (1.75 µg/kg/hr) during anaesthesia.

Score	Treatment M	Treatment D
1	1	6
2	5	3
3	0	1
4	2	0
5	2	0
6	0	0

*Significant differences between treatments were found ($p = 0.015$).

Post-anaesthetic period

One horse of treatment M developed signs of colic and pulmonary oedema (foamy nasal discharge and tachypnoea, confirmed by ultrasound) three hours after the surgical procedure. No episodes of airway obstruction were noticed during recovery. Urine production during anaesthesia was approximately eight litres. The horse was successfully treated [furosemide (Dimazon, Intervet, Belgium) (0.6 mg/kg, IV, TID); hyoscine butylbromide (Buscopan, Boehringer Ingelheim, Belgium) (0.2 mg/kg, IV, SID) and ipratropium bromide (Atrovent, Boehringer Ingelheim, Belgium) (0.7 µg/kg, via aerosol, QID)] and returned to normal condition two days after anaesthesia.

Moreover, two horses showed box-walking behaviour after receiving treatment M for up to two hours.

Discussion

In the present study, the cardiopulmonary function was maintained at clinically acceptable levels with both morphine and dexmedetomidine CRIs. A reduction of the required maintenance dose of isoflurane was demonstrated after one hour of anaesthesia in horses receiving treatment D compared to treatment M. Ketamine was needed less often and recovery qualities were better in horses in treatment D, with less attempts to stand. One horse receiving treatment M developed post-operative colic and pulmonary oedema and two more horses showed box-walking.

A first limitation of the present study was the number of horses involved. Due to differences in recovery quality between the two groups and also because of the difficulty to maintain anaesthesia in some horses receiving morphine, it was decided to discontinue the trial after the first twenty horses. Although no overall differences were found in $F_{E'}\text{ISO}$ between the two protocols, significant differences could be demonstrated in different important parameters including the cardiopulmonary function, recovery quality, additional ketamine used and the isoflurane requirements at T60, suggesting that the number of horses, and the power of the study, were sufficient to make clear end conclusions. Another limitation was the duration of anaesthesia, which was only sixty minutes in several horses. Due to the effects of premedication and induction drugs and because the initial $F_{E'}\text{ISO}$ was the same in both groups, differences in cardiopulmonary function and parameters such as $F_{E'}\text{ISO}$ may only become clear after a longer anaesthetic period. Finally, the inclusion of a placebo group would have been of interest to compare the effects of both drugs on the cardiopulmonary and $F_{E'}\text{ISO}$ values, duration of recovery and recovery scores, but this was not the primary aim of the study since a comparison of the effects of both morphine (Clark et al. 2005) and dexmedetomidine (Marcilla et al. 2012) CRIs with those of saline had already been reported in clinically healthy horses.

Dexmedetomidine (3.5 $\mu\text{g}/\text{kg}$, IV) was used for sedation. This dose was based on the study by Bettschart-Wolfensberger et al. (2005) where 7 $\mu\text{g}/\text{kg}$ of medetomidine was estimated to be equipotent to 3.5 $\mu\text{g}/\text{kg}$ dexmedetomidine. However, in a clinical study using the same dose, additional dexmedetomidine doses were required to obtain a good sedative level before induction of anaesthesia in eleven out forty horses (27.5%) (Marcilla et al. 2012). In the results presented here, a similar pattern was observed, but a

larger proportion of the horses receiving dexmedetomidine plus morphine required an extra dose of dexmedetomidine compared to horses receiving dexmedetomidine alone (60% versus 20%). Although it is difficult to draw conclusions because of the limited number of horses in the present study, these results are nevertheless surprising and in disagreement with those of Clarke & Paton (1988), who reported that the use of an opiate together with an α_2 -agonist greatly reduced the response to stimulation and improved sedation in standing horses compared to the α_2 -agonist alone. It may be hypothesized that a stimulation of the central nervous system by morphine occurred, but signs of excitement were not observed in any of the horses receiving the morphine protocol. According to these findings and to those of our previous work (Marcilla et al. 2012), an increase in the dosage of dexmedetomidine should be considered in order to obtain better level of sedation before induction of general anaesthesia.

One of the main reasons to start this clinical study was to have a comparison of the isoflurane requirements between both protocols. However, demonstrating a clear reduction in isoflurane requirements with α_2 -agonist CRIs in blinded clinical studies has shown to be difficult (Devisscher et al. 2010; Schauvliege et al. 2011; Marcilla et al. 2012). The most logical explanation for this might be that α_2 -agonists have little effect on classical parameters that are used to assess anaesthetic depth, including ocular reflexes and position of the eye. A plane of anaesthesia that would normally appear light and possibly inadequate should be tolerated during these infusions. It therefore becomes less likely that a difference in isoflurane requirements will be demonstrated in blinded clinical studies. Consequently, an adaptation of the flow-chart from Enderle et al. (2008) was used in the present study, which has been described as ‘a relatively objective tool to titrate the concentration of isoflurane delivered to the patient for the anaesthetist, unaware of the combination being administered to the horse’.

Although overall differences between treatments in $F_E \dot{V}_{ISO}$ were not present during the first sixty minutes of anaesthesia, the difference between both groups gradually became larger and was significant after sixty minutes. As explained earlier, this observation might be related to a gradual reduction of the effects of the premedication and induction drugs. After ninety minutes of anaesthesia, the difference was indeed even larger, but not significant, mainly due to the low number of horses exceeding one hour of anaesthesia. Nevertheless, the $F_E \dot{V}_{ISO}$ values of both treatments

were lower than the reported isoflurane MAC values for horses (Steffey et al. 1977, 2003), supporting the idea that analgesia and/or additional central nervous system depression was provided by both CRIs. Dexmedetomidine infusions provide sedation and/or analgesia in other species (Franken et al. 2008; van Oostrom et al. 2011) and reduced the MAC of isoflurane in ponies under experimental conditions (Gozalo-Marcilla et al., 2013a). In contrast, the demonstration of analgesia in horses receiving morphine is not so straightforward (Bennett & Steffey 2002; Clutton 2010), especially when performed in pain-free horses or ponies. Non-painful horses used in experimental studies where an electrical stimulation was applied and morphine was administered had a surprising increase of the MAC of the inhalant agent (Steffey et al. 2003), mainly due to central arousal overwhelming any analgesic action of the opioid (Bennett & Steffey, 2002). In agreement with these reports, the results of the present study confirm that, at the doses used, the isoflurane requirements are lower during a dexmedetomidine CRI than during a morphine CRI in horses.

Horses of the dexmedetomidine group spent more time at anaesthetic score 0, which was considered as the 'ideal' depth of anaesthesia. When treatment M was infused, more adjustments according to the flow-chart had to be performed. Moreover, more ketamine was needed and an optimal level of anaesthesia was more difficult to achieve since nystagmus and/or movement occurred more often. In contrast, Clark et al. (2005) concluded that horses receiving morphine tended to receive fewer and lower doses of ketamine. In the study of Clark et al. (2005), halothane was administered at one MAC whereas in this study all the horses at T10 were anaesthetized with a 0.63 to 0.69 MAC of isoflurane, according to the isoflurane MAC values of Steffey et al. (1977, 2003).

It may be argued that 0.9% isoflurane was not enough for an adequate anaesthetic maintenance when morphine was administered as a CRI, leading to a more unstable anaesthesia accompanied with intra-operative movements and additional doses of ketamine compared with treatment D. However, as soon as an F_E ISO of 0.9% was reached after induction of anaesthesia, the flow-chart was used, which allowed increases in F_E ISO when anaesthetic depth was deemed inadequate and which was primarily based on the usual signs to assess anaesthetic depth, such as palpebral reflexes, nystagmus, movement, etc. Our flow-chart was adapted from Enderle et al. (2008).

According to the latter, anaesthetic depth was never decreased in horses where a palpebral reflex was present. In our flow-chart, anaesthetic depth in such horses was decreased when arterial blood pressure decreased considerably compared to the reference value or when mean arterial pressure decreased below 70 mm Hg (despite the prescribed use of dobutamine in these cases). It may be argued that dexmedetomidine increases arterial blood pressure and therefore influenced the decision making in the flow-chart. However, higher blood pressures would rather lead to positive scores and thus prescribe an increase in anaesthetic depth, but in contrast, $F_E \text{ ISO}$ was lower during treatment D.

Cardiovascular function was maintained within physiological limits in all horses. Although arterial blood pressures were stable during both treatments, MAP and DAP were significantly lower in horses receiving morphine. Six horses from treatment M, but only three horses from treatment D required dobutamine to maintain MAP over 70 mmHg. Cardiac index and SVI were lower in horses receiving only dexmedetomidine, although these differences were of limited relevance in clinically healthy horses. Since HR was comparable between groups, the decrease in CI was mainly due to a decrease in SVI. Although direct effects of α_2 -agonists on myocardial contractility are not common (Flacke et al. 1990), a decrease in SVI might be due to a combination of an increase in SVR, which leads to an increase in afterload together with decreased sympathetic tone (Bloor et al. 1992). Moreover, this increase in SVR, although not of statistical significance, led to the higher arterial blood pressures in horses receiving dexmedetomidine, mainly due to the activation of α_{2b} -adrenoceptors on endothelial smooth muscle (Guimarães & Moura 2001). These increases in arterial blood pressures when administering dexmedetomidine are consistent with the findings of our previous clinical study (Marcilla et al. 2012).

No significant differences were found in arterial blood gases. All the horses were mechanically ventilated from the beginning of the anaesthesia in order to more easily reach the different $F_E \text{ ISO}$ values required, which could have masked potential differences in pulmonary parameters. Previous reports showed that horses receiving dexmedetomidine had significantly lower PaO_2 values compared to a control group, although DO_2I and CaO_2 were comparable in both groups (Marcilla et al. 2012). A morphine infusion had no effect on either PaCO_2 or PaO_2 (Clark et al. 2005), although

two out of six experimental ponies showed clear signs of hypoxaemia (Gozalo-Marcilla et al. 2013b).

Recovery remains a critical phase in equine anaesthesia. Better recoveries were seen in horses receiving treatment D, with less attempts to stand compared with those receiving treatment M, without significant differences in recovery times. Compared to saline, recovery qualities following a dexmedetomidine CRI were better (Marcilla et al. 2012). After a morphine infusion, recoveries were characterized by fewer attempts to attain sternal recumbency and standing, with a shorter time from the first recovery movement to the time of standing (Clark et al. 2008). The authors of that study attributed the presence of ‘quieter’ recoveries to the analgesic effects of morphine. Again, the presence of a placebo group would have allowed us to find differences in recovery quality between dexmedetomidine and morphine compared to saline. As previously stated, it could be argued that horses in treatment M received an insufficient dose of isoflurane to maintain an adequate surgical plane of anaesthesia, therefore requiring higher amounts of ketamine, which could have led to worse recoveries (Bettschart-Wolfensberger et al. 1996; Bettschart-Wolfensberger & Larenza 2007). The administration of a small dose of dexmedetomidine in order to prevent complications during the recovery period (Santos et al. 2003) might explain the absence of differences in recovery times between groups.

No post-operative complications were observed in any of the horses receiving treatment D, as previously reported (Marcilla et al. 2012). One horse showed signs of colic and pulmonary oedema after treatment M. Although it is impossible to draw appropriate conclusions from one single case, opioids have previously been shown to produce constipation in the gastrointestinal tract, causing a prolonged depression of the intestinal propulsion (Roger et al. 1985). Nevertheless, no increase in the incidence of colic was reported after IV administration of boluses (Mircica et al. 2003) or infusions of morphine (Clark et al. 2005, 2008). Alpha₂-agonists can also decrease intestinal motility, predisposing to ileus (Valverde 2010), but none of the horses of the present study, all receiving dexmedetomidine, or the twenty horses of our previous clinical study (Marcilla et al. 2012), showed any sign of intestinal discomfort. Interestingly, the horse showing signs of colic also developed clinical signs of pulmonary oedema, confirmed via ultrasound, returning to normal status in two days. Up to now, two cases

with postoperative pulmonary oedema in horses have been mentioned in literature where morphine was suggested to be a potential cause (Kaartinen et al. 2010). Although the reasons remain unclear, the authors concluded that fluid overload during anaesthesia could have been worsened by morphine-induced reduction in urine production and by potential morphine-induced changes in pulmonary permeability. In that study, the two horses produced only three and two litres of urine after total anaesthesia times of 5.3 and four hours respectively, whereas the horse affected here produced eight litres after three hours of anaesthesia, suggesting that other factors could have been involved in the development of pulmonary oedema (Senior 2005). Furthermore, two other horses receiving treatment M showed clear signs of box walking after the recovery for up to two hours. This excitatory behaviour was also present in two of six experimental ponies receiving morphine infusions (Gozalo-Marcilla et al. 2013b) and after doses as high as 2 mg/kg in experimental studies by Steffey et al. (2003), but not at lower doses (0.25 mg/kg). No box-walking behaviour was reported after morphine administration after single boluses (Mircica et al. 2003; Love et al. 2006) or as a bolus followed by a CRI (Clark et al. 2005, 2008).

In conclusion, at the doses reported here and at the starting isoflurane value of 0.9%, a dexmedetomidine CRI appeared to maintain a more stable light surgical depth of anaesthesia, with fewer and lower doses of additional ketamine and lower isoflurane requirements, especially at sixty minutes after induction of anaesthesia, compared with a morphine CRI. Furthermore, recovery qualities were significantly better with a lower number of attempts to stand in horses receiving treatment D. Post-operative complications such as pulmonary oedema, colic and box-walking were occasionally observed in morphine horses.

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General discussion

As mentioned in the introduction of this PhD, general anaesthesia in equines carries a higher risk of mortality compared with other species, volatile anaesthetic agents making the most significant contribution (Johnston et al. 2002). Consequently, the concept of equine balanced anaesthesia has been introduced. In summary, balanced anaesthesia in horses includes the combination of volatile anaesthetics with intravenous (IV) anaesthetics and/or locoregional anaesthetic techniques, in order to maintain a good intraoperative cardiopulmonary function followed by calm, smooth and coordinated recovery (Bettschart-Wolfensberger & Larenza 2007). The use of different drugs as IV constant rate infusions (CRIs) in order to reduce the amount of inhalant anaesthetic agent, and to provide additional sedation and analgesia has been reviewed in depth in the Section 1 of the general introduction. Although encouraging results have already been obtained, most commonly used drugs are accompanied with side effects. Lidocaine may produce ataxia in the recovery period and toxicosis may also occur. Excitatory side effects that can worsen the quality of the recoveries after ketamine infusions have been reported. Finally, the use of opioids in horses remains controversial and may be limited, mainly due to their inconsistent minimum alveolar concentration (MAC) reduction, central nervous system stimulation and reduced gastrointestinal motility. The search for the ideal drug combination for balanced anaesthesia in horses therefore continues.

Alpha₂-agonists are potent sedatives and analgesics and their use as CRIs during equine general anaesthesia may reduce the MAC of inhalant agents and improve the recovery qualities. Although these agonists are not free of cardiovascular side effects, which can be of major importance in compromised patients, it seems possible that the impact on cardiovascular function would be limited due to their associated MAC reducing effects. Intraoperative infusions of different α_2 -agonists have become popular and, although not licensed for use in food producing horses, medetomidine has been widely studied in anaesthetized horses. Medetomidine is an equal mixture of two optical enantiomers, with dexmedetomidine being entirely responsible for the sedative, analgesic and dose dependent anaesthetic sparing effects (Segal et al. 1988; Ansah et al. 1998; Kuusela et al. 2000). As reviewed in Section 2 of the general introduction, dexmedetomidine has been used in different species with promising results. Unfortunately, only one study described the cardiopulmonary effects and pharmacokinetics of dexmedetomidine after IV administration in experimental ponies

(Bettschart-Wolfensberger et al. 2005). In that study, it was stated that preliminary trials showed that 7 µg/kg medetomidine was equisedative to 3.5 µg/kg dexmedetomidine. Using this dose, dexmedetomidine induced similar cardiopulmonary changes compared to other α_2 -agonists, but of very short duration. The authors concluded that it is a rapidly redistributed and short-acting sedative drug with a rapid initial decline of drug concentration. Consequently, the inclusion of dexmedetomidine as part of balanced anaesthetic regimes was suggested by these investigators.

Based on the above mentioned equipotencies between medetomidine and dexmedetomidine, we examined the cardiopulmonary effects of two different CRI rates of dexmedetomidine in isoflurane anaesthetized healthy ponies in our **first experimental study**. After sedation with dexmedetomidine (3.5 µg/kg, IV) and induction, the cardiopulmonary effects of two different CRI rates (1 and 1.75 µg/kg/hr) were evaluated in each pony to test their safety during isoflurane anaesthesia. Both rates produced statistically significant cardiovascular effects, typical for α_2 -agonists (i.e. decreases in heart rate, cardiac index and oxygen delivery index). The observed side effects were of limited clinical relevance despite maintenance of anaesthesia with an end-tidal isoflurane concentration higher than one MAC.

However, there was a tendency for a lower arterial partial pressure of oxygen (PaO_2). This observation may be due to either the occurrence of hypoventilation, diffusion impairment or ventilation/perfusion (\dot{V}/\dot{Q}) mismatch and shunting or a combination of these factors. In awake horses, α_2 -agonists were demonstrated to decrease respiratory rate (Daunt & Steffey 2002), but with an increase in tidal volume (Lavoie et al. 1992), keeping the alveolar ventilation and arterial blood gas values relatively constant (Lemke 2007). Hypoventilation may still occur in anaesthetized patients, mainly due to an increase in anaesthetic depth induced by the combination of an α_2 -agonist with the volatile anaesthetic agent (Steffey et al. 2000). However, since all the ponies were mechanically ventilated, hypoventilation can be excluded as a major cause of hypoxaemia. Diffusion impairment is very unlikely since it rarely occurs in healthy animals. Consequently, the hypoxaemia noticed in these experimental ponies was most likely due to \dot{V}/\dot{Q} mismatch and shunting. It may be hypothesized that dexmedetomidine influenced pulmonary perfusion, but to the authors' knowledge, deterioration of \dot{V}/\dot{Q} matching and/or shunting have not been reported after

administration of α_2 -agonists. It is however well known that pulmonary function and oxygenation are often impaired in anaesthetized horses (Hall et al. 1968; Nyman & Hedenstierna 1989). This might be aggravated by the conformation of the ponies (Moens 1989), as they were ‘round bellied’ and relatively fat and heavy. Overall, no significant differences in the cardiopulmonary function were present between both CRI dexmedetomidine rates. Moreover, recoveries from general anaesthesia were good, with minimal ataxia. These findings might have been masked by a ‘learning effect’ as the ponies had been previously enrolled in other experimental studies.

Unfortunately, the study design did have some important limitations. Potential carry-over effects of the dexmedetomidine CRI infused during the first period of the study may have influenced the cardiopulmonary parameters of the second period, despite the washout period of thirty minutes between both periods. Although not ideal, the study design and the relatively short washout period were included to limit the number of anaesthetic procedures per pony but also to reduce the total anaesthetic time in each individual pony. Ideally, each pony would have been anaesthetized twice receiving randomly each dexmedetomidine CRI rate. As described by Bettschart-Wolfensberger et al. (2005), the cardiopulmonary effects of dexmedetomidine are short lasting, suggesting that a ‘washout period’ of thirty minutes after the end of the first CRI would have been justified. Furthermore, this assumption was confirmed by the comparable baseline values recorded at the beginning of both periods, suggesting that carry-over effects did not or minimally influenced our results.

Moreover, it can be argued that the simultaneous administration of other anaesthetic drugs might also have influenced our results and it would have been better to induce anaesthesia with only a volatile agent, as described for the determination of the MAC in most species. However, ketamine and midazolam (or other benzodiazepines) are routinely used for induction of anaesthesia in horses and their cardiovascular influences are always present under clinical situations. Moreover, although the sympathetic stimulation characteristics of ketamine can have an effect on all the values but mainly the baseline values recorded after thirty minutes of anaesthesia, these stimulating effects may have been counteracted by the dexmedetomidine’s sedative effects or even the administration of midazolam.

The results of our first study suggested that both protocols produced cardiopulmonary effects typical of α_2 -agonists, although limited and within an acceptable clinical range. In conclusion, both infusion rates are suggested to be useful in healthy, anaesthetized clinical horses.

The **second study** was a blinded clinical trial in healthy equine patients undergoing elective surgeries in which we showed that a dexmedetomidine CRI can be safely used as an adjunct during isoflurane anaesthesia. The results of the first study were indicative that both rates produced similar cardiopulmonary effects, mainly due to the reported ceiling effect of the α_2 -agonists (Pypendop & Verstegen 2001). Indeed, α_2 -agonists produce a dose related sedation and analgesia (Ansah et al. 1998; Slingsby & Taylor 1998), but beyond a certain dose only minimal changes on cardiopulmonary function can be expected. It was hypothesized that 1.75 $\mu\text{g}/\text{kg}/\text{hr}$ dexmedetomidine would possibly provide more sedation and analgesia. Reports in rats (Bol et al. 1999), cats (Slingsby & Taylor 2008) and dogs (van Oostrom 2011) showed that the sedative effects of dexmedetomidine were reached at lower doses than those required for an adequate level of analgesia. Moreover, it has been stated that analgesia cannot be produced without sedation and sedation is not necessarily linked to comparative degrees of analgesia (Franken et al. 2008). In contrast, it may be argued that comparable degrees of sedation and analgesia were obtained with both rates of dexmedetomidine in our experimental first set up. Indeed, it remains unclear whether a ceiling effect for the sedation and analgesia did occur in our experimental ponies. Although this ceiling effect has been reported in dogs (van Oostrom 2011), and suggested in cats (Ansah et al. 2000), up to now insufficient data are available to support this effect in horses and further studies are certainly justified. We preferred the higher dose as its equipotent dose of medetomidine was successfully used in horses. Medetomidine (7 $\mu\text{g}/\text{kg}/\text{hr}$) was suitable for prolonged use, providing a constant level of sedation (Bettschart-Wolfensberger et al. 1999), together with clear MAC-sparing effects (Bettschart-Wolfensberger et al. 2001; Neges et al. 2003). Additionally, a subjectively easier maintenance of a stable anaesthetic depth was reported when compared to horses receiving isoflurane alone (Neges et al. 2003).

The results of our second study demonstrated that dexmedetomidine at a dose of 1.75 $\mu\text{g}/\text{kg}/\text{hr}$ can be safely used in isoflurane anaesthetized healthy equine patients. No

clinically relevant effects on the cardiovascular system were present compared to a control group. Although PaO₂ was significantly lower in horses receiving dexmedetomidine, the mean values were over 100 mmHg and the oxygen delivery index did not differ between groups.

An important finding concerning the clinical application of dexmedetomidine was the significantly improved recovery qualities. This observation might have been related to the significantly longer times these patients remained in the sternal recumbent position and the first attempt to stand, resulting in less residual effects of the anaesthetics on the motor function and coordination. This was not seen after romifidine (Devisscher et al. 2010) or detomidine CRIs (Schauvliege et al. 2011), most likely due to the extra inclusion of a small amount of the α_2 -agonist at the end of anaesthesia, masking potential differences (Schauvliege et al. 2011). However, better recoveries were observed in our clinical study, even when a small dose of dexmedetomidine was added, just prior to recovery, in both the saline and dexmedetomidine groups. Furthermore, the number of horses may have had an influence on the obtained results as well, since the clinical dexmedetomidine study had more statistical power compared to the other two studies (Devisscher et al. 2010, Schauvliege et al. 2011). To the authors' knowledge, none of the other studies did compare the recovery qualities after a CRI medetomidine versus saline in anaesthetized horses. Horses receiving medetomidine did show better recoveries than those receiving lidocaine (Ringer et al. 2007), while medetomidine added to a lidocaine CRI improved the recovery qualities (Valverde et al. 2010).

The clinical protocol of the second study failed to show a reduction of isoflurane, which was in agreement with previous blinded studies using different α_2 -agonist CRIs (Devisscher et al. 2010; Schauvliege et al. 2011). Horses receiving a medetomidine CRI were reported to be much 'lighter' compared to sole inhalational anaesthesia (Kalchofner et al. 2006) while equine patients were clinically assessed as 'light' by the anaesthetist did not respond to noxious stimuli when medetomidine was being infused (Ringer et al. 2007). All these properties render it relatively difficult to find clear differences in the volatile requirements in a blinded study.

Due to the limitations of blinded clinical studies, an experimental MAC study was performed in experimental ponies as **third study** in order to possibly detect MAC

reductions induced by a dexmedetomidine CRI. The six ponies used in the first trial were anaesthetized twice with sevoflurane and received randomly a saline placebo or a 3.5 µg/kg dexmedetomidine bolus followed by a 1.75 µg/kg/hr CRI. The MAC of sevoflurane in our experimental ponies was $2.42 \pm 0.55\%$ which was similar to values reported in horses (Aida et al. 1999; Rezende et al. 2011). In this trial, we demonstrated that the inclusion of a dexmedetomidine CRI did significantly reduce the MAC of sevoflurane to $1.07 \pm 0.21\%$ (mean MAC reduction $53 \pm 15\%$).

In order to avoid possible interactions with other drugs, most of the equine MAC studies use a face mask delivering the volatile agent to induce anaesthesia (Aida et al. 1994; Tendillo et al. 1997; Steffey et al. 2000). In order to avoid pollution of the working area and to minimize adverse reactions of the animals during inhalation of the gas mixture, the ponies were nasotracheally intubated as described in foals (Webb 1984). To our knowledge, this is the first time that this method has been reported for MAC studies in adult ponies. However, one relatively nervous pony did panic when she became ataxic. Sedation with xylazine was needed to allow a smooth induction in this pony. It may be argued that this pony should have been excluded from the study since xylazine might have influenced MAC determinations. However, in that individual case, the first MAC determination was achieved after 120 and 300 minutes when receiving a saline or a dexmedetomidine CRI, respectively. Even though xylazine is accepted as a short acting α_2 -agonist, the sedative dose might influence the volatile anaesthetic requirements for at least three hours after injection (Steffey et al. 2000, Bennett et al. 2004). Nevertheless, exclusion of that pony did not significantly alter the overall MAC values, which were $2.43 \pm 0.62\%$ and $1.08 \pm 0.24\%$ during the placebo and dexmedetomidine CRIs respectively (mean MAC reduction $53 \pm 17\%$).

Another remark that may arise is the non-traditional use of the term MAC. We stated that ‘the response to the electrical stimuli was considered positive when a gross purposeful movement of head, limbs or tail occurred and/or swallowing or generalized muscle tremors were observed following a constant-current (CC) noxious stimulus. Gross purposeful movement without CC stimulation was also considered a positive response’. Traditionally, MAC is defined as the alveolar concentration at which 50% of the patients do not respond with purposeful movement to a supramaximal noxious stimulus (Merkel & Eger 1963). According to this, non-purposeful movement is

permissible during MAC determinations. In our study, we used a different definition because non purposeful movement would not be allowed under clinical circumstances but also because differentiation between purposeful and non-purposeful movements remains confusing, making this process somewhat subjective (Seddighi et al. 2011). In fact, the definition applied in the present PhD fits better with the definition of the more recently proposed term ‘MAC-no movement’ (MAC_{NM}) which has been formulated as the lowest alveolar concentration of an anaesthetic that abolishes all movement (Seddighi et al. 2011, 2012). Indeed, the determination of MAC_{NM} instead of MAC was suggested to reduce the subjectivity of classic MAC studies and is more clinically relevant. In retrospect and taking the recent literature into account, the use of the term MAC_{NM} in both of our MAC studies would have been more appropriate.

It is well known that the use of morphine remains controversial in equine anaesthesia (Bennett & Steffey 2002; Clutton 2010), and administration of IV boluses produced inconsistent changes in the MAC requirements of isoflurane (Steffey et al. 2003). Although there was a tendency in clinical anaesthetized equine patients receiving a morphine CRI to require fewer and lower doses of additional anaesthetic drugs (Clark et al. 2005), the effects of a morphine CRI on the MAC_{NM} of volatile agents had not been reported. Due to the positive findings of our previous study and because the protocol was deemed suitable for use in further studies, we aimed to determine the effects of a morphine CRI on the MAC_{NM} of sevoflurane. Moreover, we hypothesized that the co-administration of a dexmedetomidine CRI may influence the MAC_{NM} values, probably reducing them.

In the **fourth study**, five experimental ponies were anaesthetized with sevoflurane, as described in the third study, and they received randomly the morphine protocol described by Clark et al. (2005) (IV bolus 0.15 mg/kg followed by CRI at 0.1 mg/kg/hr), alone or combined with our dexmedetomidine protocol. When receiving a CRI of morphine, the MAC_{NM} sevoflurane value was determined to be $2.79 \pm 0.73\%$, whereas co-infusion of dexmedetomidine significantly reduced that value to $0.89 \pm 0.22\%$ (mean MAC_{NM} reduction $67 \pm 11\%$). The pony which needed sedation before induction of anaesthesia in the third study was excluded due to potential influences in the MAC_{NM} determination.

General discussion

The most important limitation of the fourth study was the absence of a control, placebo group. Ideally, one larger MAC_{NM} study should have been performed, with each pony receiving the four treatments in a randomized order. Although both experiments were performed in the same ponies and within a period of five months, a direct statistical comparison between all four treatments was not made since the order of the treatments was not randomized, and the main anaesthetist was not fully blinded. In the author's opinion, it was however deemed unethical to repeat the MAC_{NM} determinations for the dexmedetomidine and saline treatments in these ponies. Although the MAC_{NM} values obtained in the two studies are of value for further research, the only conclusion that can be made from our independent studies is that a CRI of dexmedetomidine significantly reduces sevoflurane's MAC_{NM} values in ponies when added to saline or morphine infusions.

A study including the four treatments might also have determined if a morphine infusion increases MAC_{NM} values of sevoflurane and/or if the addition of both dexmedetomidine and morphine further reduces the MAC_{NM} values compared to dexmedetomidine alone. However, although a direct statistical comparison between the four groups was not possible, it still remains of scientific interest to note that the MAC tended to increase when a CRI of morphine was infused, increasing the MAC of sevoflurane from $2.42 \pm 0.55\%$ to $2.79 \pm 0.73\%$. At the same time, combination of both morphine and dexmedetomidine infusions further reduced the sevoflurane's MAC compared to dexmedetomidine alone from $1.07 \pm 0.21\%$ to $0.89 \pm 0.22\%$. Under clinical circumstances, co-administration of both CRIs would be of interest in order to reduce the MAC and potential side effects of the volatile anaesthetic, as dexmedetomidine seemed to alter the effect of morphine on MAC requirements.

Interesting observations were made when morphine was administered as CRI compared to saline or dexmedetomidine alone. It is worth mentioning that the responses to the stimuli were different compared with the findings of the third study. Maintenance of anaesthesia was judged to be more difficult in two out of five ponies when receiving the morphine treatment, with the occurrence of generalized, almost uncontrollable muscle tremors, and 'fighting' against the ventilator. These differences were explained in the discussion by a combined analgesic, excitatory and locomotor stimulant effects attributed to the use of morphine in horses (Kamerling et al. 1989). Moreover, two

ponies showed after the morphine CRI a typical ‘box-walking’ behaviour for up to two hours after the end of anaesthesia, one of them with clear over-reactions to external stimuli. In both circumstances, co-infusion of dexmedetomidine seemed to avoid such reactions, most likely due to its sedative properties. Moreover, prolonged infusions of morphine with dexmedetomidine shortened the recovery times, which can possibly be attributed to the effects of morphine accumulation after prolonged CRIs.

Finally, it has to be stated that the data obtained from the second MAC study should be interpreted carefully as stimulation of the central nervous system may result in MAC increases (Miller et al. 1968; Johnston et al. 1972). In the case of morphine, increases in MAC_{NM} can be induced by the central arousal overwhelming analgesic actions, which do not confirm the absence of analgesic effects (Bennett & Steffey 2002). Moreover, electrical stimulation is probably qualitatively different from classic surgical nociception (Clutton et al. 2010). Although the demonstration of analgesia in horses receiving morphine is not so straightforward (Bennett & Steffey 2002; Clutton, 2010), we hypothesized that in presence of surgical pain, morphine would exert more analgesic effects rather than producing central arousal, even producing MAC reductions. This assumption was based on previous encouraging reports with morphine infusions under clinical circumstances in horses (Mircica 2003; Clark et al. 2005, 2008; Love et al. 2006) and was the original idea to perform the following study.

In order to compare both dexmedetomidine and morphine CRIs under clinical circumstances, we performed our **fifth clinical trial** to evaluate the influence on cardiopulmonary function, intraoperative anaesthetic stability and recovery quality in equine patients. With a dexmedetomidine CRI it was possible to maintain a more stable light surgical depth of anaesthesia, requiring less isoflurane or additional drugs. Furthermore, recovery qualities were significantly better, with a lower number of attempts to stand in horses receiving dexmedetomidine. Post-operative complications such as pulmonary oedema, colic and box-walking were only observed in horses receiving a morphine CRI.

In this blinded, clinical trial not only a comparison between treatments with regard to the cardiopulmonary function and the recovery qualities was made. Moreover, the authors aimed to determine which protocol required less isoflurane and produced a more stable plane of anaesthesia. Although the MAC_{NM} of sevoflurane was higher in

our ponies when receiving a morphine CRI compared to dexmedetomidine, clinical studies showed that horses receiving the same protocol tended to receive fewer and lower doses of additional anaesthetics compared to a control group (Clark et al. 2005). As stated earlier, it remains difficult to detect potential MAC reductions when infusions of α_2 -agonists are used in blinded clinical studies.

In order to overcome these drawbacks, we designed a flow-chart adapted from Enderle et al. (2008) described as ‘a relatively objective tool to titrate the concentration of isoflurane delivered to the patient for the anaesthetist, unaware of the combination being administered to the horse’. The application of this flow-chart was successfully performed by the main blinded anaesthetist. However, anaesthesia was easier to maintain at an ‘ideal’ plane, with less intraoperative movements in horses receiving a dexmedetomidine infusion. Moreover, less isoflurane and extra anaesthetic drugs were required. These results seem to support strongly the use of a dexmedetomidine versus a morphine infusion. Nevertheless, the results of this clinical study should be interpreted carefully. It may be discussed that the study design was not ideal, mainly because of the commencing maintenance of anaesthesia at an expiratory fraction of isoflurane ($F_E \text{ ISO}$) of 0.9%. This value may have been sufficient when dexmedetomidine but not when morphine was infused, resulting in early arousal and a subsequently unstable plane of anaesthesia in the morphine group. Moreover, it cannot be excluded that central arousal effects might have been present on top of morphine’s analgesic properties, leading to intraoperative movements. Nevertheless, the flow-chart, did allow to increase the $F_E \text{ ISO}$ when the classical parameters used to evaluate anaesthetic depth in horses indicated a superficial plane of anaesthesia. In the authors’ opinion, this protocol therefore mimicked a clinical situation. In any case, when applying this relatively objective flow-chart we concluded that, when a dexmedetomidine CRI was administered, lower amounts of volatile agents are required to maintain a more stable and reliable plane of anaesthesia compared to a morphine CRI under clinical circumstances.

Only twenty equine patients were included in this last clinical trial. Although the inclusion of forty horses was initially planned, the authors decided to stop the study after twenty animals as the overall maintenance of anaesthesia in horses receiving morphine was difficult, creating problems to the surgical team. Moreover, recovery

qualities were far from ideal with one horse developing colic and even pulmonary oedema after morphine infusion. However, it remains difficult to determine whether morphine was responsible for these symptoms. Although no overall differences were found in $F_E\text{ISO}$ between the two protocols, significant differences could be demonstrated in important parameters including cardiopulmonary function, recovery quality, additional ketamine and the isoflurane requirements at T60, suggesting that the number of horses was sufficient to make clear end conclusions. Co-administration of both dexmedetomidine and morphine infusions may have led to better outcomes, providing the analgesic effects of morphine while avoiding the morphine side effects by the sedative properties of dexmedetomidine.

An interesting finding of several of these studies is that the PaO_2 values often tended to be lower when administering a dexmedetomidine CRI. Up to date, there is not a clear explanation for this. Differences in minute ventilation seem less likely, especially since mechanical ventilation was used in most horses and PaCO_2 values were comparable between groups. It may be hypothesized that dexmedetomidine influences the hypoxic pulmonary vasoconstriction (HPV) reflex, a highly efficient mechanism in the pony that distributes blood flow from hypoxic regions in the lung to ventilated areas (Elliott et al. 1991). To the author's knowledge, no studies are available on the effects of α_2 -agonists on the HPV reflex. However, it seems possible that administration of dexmedetomidine elevated pulmonary arterial pressure, disturbing the HPV mechanism. This would contribute to impaired arterial oxygenation, which was reported in horses after detomidine sedation (Nyman et al. 2009). In the latter study, significant reductions in blood flow and an increase in \dot{V}/\dot{Q} mismatch were the major contributors to the alveolar-arterial oxygen tension difference. In contrast, studies in humans showed that a bolus of dexmedetomidine followed by a CRI did not adversely affect oxygenation during one-lung ventilation (Kernan et al. 2011). In fact, the authors concluded that its use as part of a balanced anaesthetic technique may improve oxygenation by allowing the use of lower concentrations of the inhaled agent, thereby limiting its effects on oxygenation (Marshall et al. 1984; Eisenkraft 1990). In our clinical study (Chapter 2), the $F_E\text{ISO}$ was comparable between treatments ($\pm 1.1\%$). After determining that a dexmedetomidine CRI reduces the MAC_{NM} of inhalants (Chapter 3) and that general anaesthesia can be well maintained at $F_E\text{ISO}$ values close to 0.9% (Chapter 5), it seems

of interest to examine the hypothesis of Kernan et al. (2011), comparing oxygenation in horses anaesthetized with different concentrations of inhaled agents while using a dexmedetomidine CRI. Furthermore, research investigating the effects of dexmedetomidine on the HPV reflex may be of value.

Another timely point is the reliability of the method used to measure cardiac output (\dot{Q}_t). In Chapters 1, 2 and 5 \dot{Q}_t was measured by means of the lithium dilution method (LiDCO), a minimally invasive and accurate technique (Kurita et al. 1997; Linton et al. 1997) suitable for use in horses (Linton et al. 2000). However, a recent study showed that different drugs may influence the accuracy of the LiDCO sensor *in vitro* (Ambrisko et al. 2013). Therefore, although the LiDCO dilution technique was considered a valid method at the time of designing/performing these studies, the results should be interpreted carefully as potential interactions may occur. From the drugs used in our studies, ketamine may have interacted with the LiDCO sensor *in vivo*, dexmedetomidine seemed unlikely to cause bias and midazolam probably did have minimal or no interaction. However, although the results of Ambrisko et al. (2013) may be of clinical relevance, further research is required to confirm this hypothesis *in vivo*.

Based on the general results of this PhD thesis, it can be concluded that a dexmedetomidine CRI (1.75 $\mu\text{g}/\text{kg}/\text{hr}$) can be safely used in healthy anaesthetized horses, with minimal cardiopulmonary effects. Moreover, it improves the recovery quality and reduces the MAC_{NM} of inhalant agents. Dexmedetomidine can also be combined with a morphine CRI, reducing the MAC_{NM} of the volatile agent and theoretically preventing unwanted phenomena linked to the infusion of morphine. Finally, a dexmedetomidine CRI produced, under clinical circumstances using the design of our last study, a more stable anaesthetic depth, with less isoflurane requirements and better recoveries compared to a morphine CRI. Post-operative complications were more frequently seen in horses receiving morphine. Based on these results, the use of an infusion of dexmedetomidine seems to be promising from a clinical point of view, especially during surgical procedures requiring the use of a short-acting sedative, with MAC-sparing effects and better recoveries (i.e. long orthopaedic procedures such as limb fracture repair). Limiting factors include the absence of a license for this drug in horses, which reduces its use to ‘non food producing animals’ and the higher economic costs compared with other registered α_2 -agonists.

General discussion

Apart from these limitations, the results presented here are encouraging for further research. Dexmedetomidine and morphine CRIs may be combined in clinically healthy equine patients, providing analgesia by two different receptors, while the sedative effects of dexmedetomidine may reduce the potential side effects of morphine. Another direction for further investigation is the use of our protocol in compromised patients such as colic horses. As previously discussed, α_2 -agonists produce an important impact on cardiovascular function. However, combination with drugs such as phosphodiesterases III inhibitors may be beneficial, as they reduce the systemic vascular resistance and increase the cardiac output. The use of milrinone (Muir 1995) and enoximone (Schauvliege et al. 2007, 2008, 2009) has been already studied in horses and the simultaneous infusion with dexmedetomidine seems to be attractive and may be of benefit in colic horses.

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Summary

It is well known that anaesthesia in horses carries a higher risk of mortality compared to other species. The different mortality rates reported in literature have been included in the **general introduction**. Overall, maintenance of general anaesthesia with volatile agents anaesthetics carries a higher risk of death compared with total intravenous anaesthetic protocols, mainly due to the cardiovascular depressant effects of the volatile agents. The different principles to treat cardiovascular depression during general anaesthesia in horses were also highlighted, introducing the concept of 'balanced anaesthesia'.

The **Section 1** of the general introduction includes a review of the different intravenous (IV) drugs that are commonly used as constant rate infusions (CRIs) in combination with volatile agents in anaesthetized horses. Lidocaine, ketamine and opioids can be administered in order to provide analgesia or to reduce the minimum alveolar concentration (MAC) of volatile anaesthetics, although their use is not free of side effects. Alpha₂-agonists are potent sedatives and analgesics reducing the MAC of inhalant anaesthetics; the main concern is their impact on cardiovascular function. The pharmacokinetics of the most selective α_2 -agonists, medetomidine and dexmedetomidine, seem to favour their use in CRIs, mainly because of their very short lasting induced cardiopulmonary changes.

An in depth review of the characteristics and use of dexmedetomidine was included in the **Section 2** of the general introduction. This drug has been used not only in human but also in different veterinary species during inhalational anaesthesia, with promising results. Unfortunately, only one study investigated the use of dexmedetomidine in ponies, suggesting its use as a CRI for equine anaesthesia.

The objectives of the PhD were defined in the **scientific aims**. In summary, we aimed to determine a safe dexmedetomidine dose(s) which was administered as sedative before induction of anaesthesia and as a CRI in combination with volatile agents during anaesthesia. A second aim was to determine the influence of dexmedetomidine on the MAC of sevoflurane when given alone or in combination with a morphine CRI. Finally, the third goal was to compare our proposed dexmedetomidine protocol with a morphine CRI under clinical circumstances in equine patients.

The cardiopulmonary effects of two different CRIs of dexmedetomidine (1 and 1.75 $\mu\text{g}/\text{kg}/\text{hr}$) were evaluated in six ponies in a prospective, randomized, experimental

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study and incorporated in **Chapter 1**. After sedation with dexmedetomidine (3.5 µg/kg, IV) and induction with IV ketamine and midazolam (T0), anaesthesia was maintained with isoflurane [expiratory fraction of isoflurane (F_E ISO) 1.50 %] in an oxygen/air mixture (55% O₂) for 150 minutes. Three ponies received dexmedetomidine CRIs of 1 and 1.75 µg/kg/hr from T30 to T60 and T90 to T120 respectively. In the other three ponies, the order was reversed.

Heart rate (HR), cardiac index (CI), arterial oxygen content (CaO₂), venous oxygen content and oxygen delivery decreased significantly, whereas systemic vascular resistance, systolic arterial pressure and right arterial pressure significantly increased with both rates. No major differences were found between the two rates. It was concluded that although significant, the changes produced by both CRI rates of dexmedetomidine were small and within an acceptable clinical range.

Since dexmedetomidine was found to be useful with minimal cardiopulmonary effects in healthy anaesthetized ponies, the use of the highest CRI rate (1.75 µg/kg/hr) was further investigated in equine patients under clinical circumstances and included in **Chapter 2**. The inclusion of this rate was justified not only because of the 'ceiling effect' of dexmedetomidine with regard to the cardiopulmonary function, but also due to a dose dependent increase in sedative and analgesic effects. A prospective, randomized, blinded, clinical study was performed to investigate the influence of a CRI of dexmedetomidine (1.75 µg/kg/hr) on the cardiopulmonary function and recovery quality in forty healthy, isoflurane anaesthetized horses undergoing elective surgery. All the horses were sedated with dexmedetomidine (3.5 µg/kg, IV) while anaesthesia was induced with IV ketamine and midazolam and maintained with isoflurane in 55-60% oxygen. The horses were randomly allocated to receive either a CRI of dexmedetomidine (1.75 µg/kg/hr) or saline. The main anaesthetist was unaware of the treatment. All the horses received a small dose of dexmedetomidine (0.875 µg/kg, IV) at the end of anaesthesia before the recovery period.

Statistically significant decreases in HR and arterial partial pressure of oxygen (PaO₂) were found in horses receiving a dexmedetomidine CRI. However, the decrease in HR was minimal, and no periods of severe bradycardia were observed. Despite this decrease, CI was not affected and was not significantly different between treatments. Although PaO₂ was lower in horses receiving dexmedetomidine, it remained in the

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range to fully saturate hemoglobin, with a comparable CaO_2 between groups. Apart from the influence on the cardiopulmonary function, a dexmedetomidine CRI did not reduce the $F_E \dot{V}_{\text{ISO}}$ required to maintain anaesthesia but improved the recovery quality, with fewer attempts to stand and significantly longer times to sternal position and first attempt to stand.

In order to prove a possible inhalant anaesthetic-sparing effects of the proposed dexmedetomidine protocol, a classic MAC study was performed (**Chapter 3**) using six healthy ponies in a prospective, randomized, crossover, blinded experimental study. Each pony was anaesthetized twice within a washout period of three weeks for either the dexmedetomidine protocol (3.5 $\mu\text{g}/\text{kg}$ IV followed by a CRI at 1.75 $\mu\text{g}/\text{kg}/\text{hr}$) or the treatment saline. Induction of anaesthesia was performed with sevoflurane in oxygen by a nasotracheal tube and maintained with sevoflurane in 55% oxygen. Afterwards (T0, positioning on surgical table), the ponies received the appointed treatment and the MAC of isoflurane was determined after sixty minutes using a classic bracketing technique including constant-current electrical stimuli applied at the skin of the lateral pastern region. Triplicate estimations of the 'MAC-no movement' (MAC_{NM}) were obtained and averaged in each pony. The results obtained from this study showed that a dexmedetomidine CRI (1.75 $\mu\text{g}/\text{kg}/\text{hr}$) significantly reduces the mean \pm SD sevoflurane MAC_{NM} from 2.42 ± 0.55 to $1.07 \pm 0.21\%$ (mean MAC_{NM} reduction $53 \pm 15\%$).

In view of the successful outcome and positive results obtained from the first MAC study, a second MAC study was additionally performed. The study included in **Chapter 4** aimed to determine the MAC_{NM} of sevoflurane during a morphine CRI or a combination of both morphine and dexmedetomidine CRIs. The sevoflurane MAC_{NM} in five experimental ponies using a morphine CRI was $2.79 \pm 0.73\%$ while simultaneous infusion of dexmedetomidine significantly reduced sevoflurane MAC_{NM} to $0.89 \pm 0.22\%$ (mean MAC_{NM} reduction $67 \pm 11\%$).

Finally, a prospective, blinded, clinical study involving twenty horses undergoing elective surgeries was performed and reported in **Chapter 5**. Two different balanced anaesthetic protocols, dexmedetomidine and morphine CRIs, were studied with regard to cardiopulmonary function, isoflurane requirements and recovery qualities. Horses were randomly allocated to receive dexmedetomidine (3.5 $\mu\text{g}/\text{kg}$ IV followed by a CRI at 1.75 $\mu\text{g}/\text{kg}/\text{hr}$) or morphine (dexmedetomidine 3.5 $\mu\text{g}/\text{kg}$ plus 0.15

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mg/kg morphine IV followed by a CRI of morphine 0.1 mg/kg/hr). Anaesthesia was induced in both groups with ketamine and midazolam and maintained with isoflurane in 55-60% oxygen. In order to determine the isoflurane requirements during both protocols, a minimal $F_E \text{ ISO}$ of 0.9% was used after induction of anaesthesia, and then adjusted by the anaesthetist to maintain a light surgical plane of anaesthesia, according to an objective flow-chart.

Cardiopulmonary function remained stable in both groups. When the anaesthetized horses received a CRI of dexmedetomidine, less additional ketamine was required, $F_E \text{ ISO}$ was lower after one hour and more time was spent in an 'ideal plane' of anaesthesia, together with better recoveries. Some complications were observed after infusion of morphine (one post-operative colic and pulmonary oedema and two box-walking behaviours). In summary, a dexmedetomidine CRI in equine patients under clinical circumstances produced a more stable anaesthetic depth, with reduced isoflurane requirements and better recoveries, but also without post-operative complications compared with a morphine CRI.

As an overall conclusion it can be stated that a dexmedetomidine CRI (1.75 $\mu\text{g/kg/hr}$) can be safely used in healthy anaesthetized horses with minimal cardiopulmonary effects, producing a reduction of the isoflurane requirements, an easier maintenance of a stable plane of anaesthesia and longer but better recoveries. This dexmedetomidine protocol was also proven to be better compared to a morphine CRI in equine anaesthetized patients.

Samenvatting

Het risico van algemene anesthesie is beduidend hoger bij paarden dan bij andere diersoorten. Verschillende mortaliteitspercentages werden in de literatuur reeds gerapporteerd, zoals beschreven in de **algemene inleiding**. Onderhoud van algemene anesthesie met volatiele anesthetica houdt bovendien een hoger risico in vergeleken met totaal intraveneuze anesthesie, vooral omwille van de meer uitgesproken cardiovasculaire depressie die optreedt. De verschillende principes die belangrijk zijn bij de behandeling van cardiovasculaire depressie werden bijkomend besproken, waarbij ook aandacht werd gegeven aan het concept van ‘balanced anaesthesia’.

Sectie 1 van de algemene inleiding geeft een overzicht van de verschillende intraveneuze (IV) farmaca die gebruikt worden als continu infuus (CRI) in combinatie met volatiele anesthetica tijdens algemene anesthesie bij paarden. Lidocaïne, ketamine en opioïden kunnen toegediend worden om analgesie te bekomen en tevens om de minimum alveolaire concentratie (MAC) van inhalatie-anesthetica te verlagen, alhoewel ze niet vrij zijn van neveneffecten. Alfa₂-agonisten zijn krachtige analgetica en verlagen de MAC van inhalatie-anesthetica, maar hebben zelf ook een duidelijke impact op het cardiovasculair systeem. De farmacokinetische eigenschappen van de meest selectieve α_2 -agonisten, medetomidine en dexmedetomidine, lijken gunstig voor gebruik in CRIs, vooral ook omdat de cardiopulmonaire effecten van zeer korte duur zijn.

Een overzicht van de karakteristieken en de toepassingen van dexmedetomidine wordt gegeven in **de Sectie 2** van de algemene inleiding **2**. Dit product werd reeds gebruikt bij zowel mens als dier tijdens inhalatie-anesthesie, met veelbelovende resultaten. Tot heden is slechts één wetenschappelijke publicatie voorhanden die het gebruik van dexmedetomidine bij paarden onderzocht. De auteurs suggereerden het gebruik van dexmedetomidine als een CRI bij paarden.

Dit leidde tot het formuleren van de wetenschappelijke doelstellingen van dit doctoraat. Allereerst werd er gezocht naar dosissen waaraan dexmedetomidine veilig gebruikt kan worden als sedativum voor de premedicatie en als CRI tijdens het onderhoud van een algemene anesthesie met volatiele anesthetica. Daarnaast werd bepaald of dexmedetomidine de MAC van sevofluraan beïnvloedt, zowel alleen als in combinatie met een morfine-infuus. Tenslotte werd het protocol met dexmedetomidine vergeleken met een morfine-infuus tijdens anesthesie bij paarden onder kliniekomstandigheden.

In **Hoofdstuk 1** werden de cardiopulmonaire effecten van twee verschillende dexmedetomidine CRI's (1 en 1.75 µg/kg/hr) onderzocht in zes pony's in een prospectieve, gerandomiseerde, experimentele studie. Na sedatie met dexmedetomidine (3.5 µg/kg, IV) en inductie met ketamine en midazolam (T0), werd de anesthesie onderhouden met isofluraan [expiratoire fractie (F_E ISO) 1.50 %] in een zuurstof/lucht mengsel (55% O₂) gedurende 150 minuten. Drie pony's kregen dexmedetomidine CRI's aan 1 en 1.75 µg/kg/hr van T30 tot T60 en T90 tot T120 respectievelijk. Bij de drie andere pony's werd de volgorde van beide infusiesnelheden omgewisseld. De hartfrequentie (HR), het hartdebiet (CI), de arteriële (CaO₂) en veneuze zuurstofgehalten en de zuurstofvoorziening (DO₂) daalden significant, terwijl de systemisch vasculaire weerstand, systolische arteriële druk en rechter atriale druk significant stegen met beide infusiesnelheden. Geen verschillen werden echter gevonden tussen de beide infusiesnelheden. Er werd besloten dat hoewel significant, de effecten van beide CRI's klein en klinisch aanvaardbaar waren.

Eens er vastgesteld was dat dexmedetomidine bij gezonde pony's kan gebruikt worden tijdens de anesthesie, met slechts minimale cardiopulmonaire effecten, werd in **Hoofdstuk 2** de hogere infusiesnelheid (1.75 µg/kg/hr) onder kliniekomstandigheden onderzocht bij paarden. De keuze voor deze snelheid was voornamelijk omwille van het zogenoemde 'ceiling effect' i.v.m. de cardiopulmonaire functie, terwijl de sedatieve en analgetische effecten mogelijks toch dosisgerelateerd zijn. Een prospectieve, gerandomiseerde, blinde, klinische studie werd uitgevoerd bij veertig volwassen en gezonde paarden aangeboden voor electieve chirurgie, met als doel de invloed van een dexmedetomidine CRI (1.75 µg/kg/hr) op de cardiopulmonaire functie en recoverykwaliteit na te gaan tijdens isofluraan anesthesie. Alle paarden werden gesedeerd met dexmedetomidine (3.5 µg/kg, IV) en de anesthesie werd geïnduceerd met ketamine en midazolam en onderhouden met isofluraan in 55-60% zuurstof. De paarden kregen at random een CRI van dexmedetomidine (1.75 µg/kg/hr) of fysiologische zoutoplossing als placebo. Voor de recovery werd aan alle paarden een kleine dosis dexmedetomidine (0.875 µg/kg, IV) toegediend. Statistisch significante dalingen in de HR en arteriële zuurstofspanning (PaO₂) werden gevonden bij paarden die een dexmedetomidine CRI kregen. De daling in HR was echter minimaal en er waren bij geen enkel paard periodes van ernstige bradycardie. Ondanks deze daling verschilde de

CI ook niet tussen de twee groepen. Alhoewel de PaO₂ lager was bij paarden die dexmedetomidine kregen, bleef deze steeds hoog genoeg om een volledige saturatie van haemoglobine met zuurstof te bekomen en was de CaO₂ vergelijkbaar tussen de twee groepen. Er werd geen daling gevonden in de F_EISO, maar dexmedetomidine verbeterde wel de recoverykwaliteit, met minder pogingen om recht te staan en significant langere tijden tot sternale decubitus en de eerste poging tot rechtstaan.

Om aan te tonen dat dexmedetomidine de behoefte aan inhalatie-anesthetica bij paarden vermindert, werd een klassieke MAC studie uitgevoerd (**Hoofdstuk 3**). Zes gezonde pony's werden opgenomen in een prospectieve, gerandomiseerde, blinde, experimentele crossover-studie. Elke pony werd 2 keer onder anesthesie gebracht met een tussentijd van drie weken en kreeg een infuus van ofwel dexmedetomidine (3.5 µg/kg IV gevolgd door een CRI aan 1.75 µg/kg/hr) ofwel fysiologische zoutoplossing. Inductie en onderhoud van de anaesthesie gebeurde met sevofluraan in een zuurstofrijk gasmengsel, toegediend via een nasotracheale tube. Na het plaatsen op de chirurgietafel (T0) werd het respectievelijke infuus gestart en vanaf T60 werd de MAC-bepaling begonnen met een klassieke 'bracketing' benadering, d.m.v. elektrische stimuli van een constante stroomsterkte toegediend t.h.v. de laterale kootregio. Drievoudige bepalingen van de 'MAC-no movement' (MAC_{NM}) werden bekomen en het gemiddelde per pony berekend. De resultaten van deze studie toonden aan dat een dexmedetomidine CRI (1.75 µg/kg/hr) significant de MAC_{NM} van sevofluraan doet dalen van 2.42 ± 0.55 naar 1.07 ± 0.21% (gemiddelde MAC_{NM} reductie 53 ± 15%).

Gezien het succesvol uitvoeren van en de positieve resultaten bekomen met de voorafgaande studie, werd een tweede MAC studie uitgevoerd (**Hoofdstuk 4**), met als doel de MAC_{NM} van sevofluraan te bepalen tijdens toediening van een morfine-infuus, al dan niet gecombineerd met dexmedetomidine. Vijf pony's werden gebruikt bij deze studie, die uitgevoerd werd zoals in Hoofdstuk 6. De MAC_{NM} van sevofluraan was 2.79 ± 0.73% tijdens een morfine-infuus, maar deze daalde tot 0.89 ± 0.22% wanneer ook dexmedetomidine als infuus werd gegeven (gemiddelde MAC_{NM} reductie 67 ± 11%).

Tot slot werd in **Hoofdstuk 5** een prospectieve, blinde, klinische studie uitgevoerd op twintig paarden tijdens electieve chirurgie, met als doel twee protocols voor 'balanced anaesthesia' met elkaar te vergelijken, i.e. dexmedetomidine en morfine CRI's, m.b.t de cardiopulmonaire functie, isofluraan behoefte en recovery kwaliteit. De

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paarden werden at random toegewezen aan behandeling dexmedetomidine (3.5 µg/kg IV + CRI aan 1.75 µg/kg/hr) of morfine (dexmedetomidine 3.5 µg/kg plus 0.15 mg/kg morfine IV, gevolgd door een morfine CRI 0.1 mg/kg/hr). In beide groepen werd de anesthesie geïnduceerd met ketamine en midazolam en onderhouden met isofluraan in 55-60% zuurstof. Om na te gaan of de isofluraanbehoefte met deze behandelingen beïnvloed wordt, werd na inductie van de anesthesie een minimale $F_E \text{ ISO}$ van 0.9% gebruikt, die verder aangepast werd aan de hand van een objectieve flow-chart om een zo licht mogelijke maar toch chirurgische diepte van de anesthesie te bereiken.

Met beide infusen werd een goede cardiopulmonaire functie bekomen. Bij de paarden die dexmedetomidine kregen was er echter minder ketamine nodig, was de $F_E \text{ ISO}$ na één uur anesthesie lager en werd een groter percentage van de tijd in een 'ideale' diepte van anesthesie doorgebracht, met finaal een betere recovery. Een aantal complicaties traden op bij de groep waar morfine toegediend werd (één koliek en longoedeem, twee andere 'box-walking'). De conclusie van deze studie was dat een dexmedetomidine CRI een stabielere diepte van de anesthesie geeft, met lagere isofluraanbehoeftes en een betere recovery, zonder postoperatieve complicaties.

Als een algemene conclusie kan gesteld worden dat een dexmedetomidine CRI (1.75 µg/kg/hr) veilig gebruikt kan worden, met minimale cardiopulmonaire effecten bij gezonde paarden tijdens algemene anesthesie en een lagere behoefte aan isofluraan, waarbij makkelijker een stabiele diepte van de anesthesie kan aangehouden worden. De recovery is langer maar wel beduidend beter. Dit protocol blijkt ook gunstiger ten opzichte van de combinatie isofluraan en een morfine-infuus.

Curriculum Vitae

Curriculum Vitae

Miguel Gozalo Marcilla was born the 13th of August of 1981 in Palencia, Spain. After finishing his secondary studies, he started in 1999 the studies of veterinary medicine at the University of León. He spent four years in León, with an intermediate academic year (2002-2003) at the UTAD of Vila Real, Portugal (Erasmus Programme) and graduated as a veterinarian in December 2004.

His interest in horses became clear when he was a child and later when he was a student at the university. After his studies, he decided to do an one year internship at the equine section of the Hospital Veterinario Sierra de Madrid (HVSM) where he started to focus on anaesthesia. After completing a training period of two months in the Dierenkliniek de Bosdreef in equine anaesthesia, he returned to the HVSM where he worked as a veterinary assistant for nine months. The Faculty of Veterinary Medicine of the University of Ghent gave him the opportunity to start a residency in anaesthesia and analgesia in September 2007. After completion of the four years residency, he passed the written examination of the European College of Veterinary Anaesthesia and Analgesia (ECVAA) and will sit the oral part in the near future. During his residency, he started to work on the topic of his PhD research, the use of dexmedetomidine for balanced anaesthesia in horses. He continued his work for another two more years as a PhD student (Dehousse grant), combining research with clinical and educational tasks.

Miguel Gozalo Marcilla is author and co-author of several papers published in international peer reviewed journals. His work has also been presented at different international congresses.

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When visiting other places you can see that sometimes the relationship between surgeon and anaesthetist is not great. I cannot say the same in our department. I would like to thank Professors Ann Martens, Lieven Vlaminck and Frederik Pille for their trust on my work. As an anaesthetist it is really important to feel that the surgeon appreciates your job and relies on your decisions. Working with all of you I felt very confident. Moreover, I would like to mention to all the surgery residents I have worked with: Jeroen (all the best in your job), Tamara (impossible to forget ☺), Mireia (good luck with your PhD), Helga (my best wishes for your near future and in your next steps) and Thomas (all the best in your residency). Caroline, take care of all the calves with 'spastische parese' as well as Marteen does it with the sarcoids. Maarten and Michèle, thanks for your help at the beginning of my residency during your anaesthesia duties. The same for Geert and Annelies, now with other positions. Kelly, I met you when you

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One of the aims of the residency and PhD is to be able to perform research, to obtain results and present them to other colleagues in international meetings. These

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During my stay in Belgium I met lots of people, some of them are now my friends. In a special place are those I met during my first year (in their Erasmus), when they were only little “polluelos” that had to learn to fly. We spent great moments together, Gentse Feesten, the trips to Normandy, Valladolid, Bériz, Cantabria, Nijmegen, and other places to make the “gañán”. We will meet again for sure (several times, chacho). Thanks Kike, Celia, Inés, Adri, Dani-Agorer, Kachenka and Peñu for making my first year in Ghent easier.

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I thought that all the people from Andalucía were very funny, making good jokes. I met two exceptions in Belgium. In Liège I met the most successful veterinary surgeon performing ‘spinal disc herniations’, un gitanillo de Málaga. Juanmi, disfruta del buen tiempo del sur y suerte con el examen! Here, in Merelbeke, I met my good friend Alfonso. Foske, try to follow as usual, giving ‘negative’ comments to the people and trying to defend that Sevilla FC is the best team ever. Take care of Evelyne, Mireille and your ‘cachorros’. Alfonso made possible that Rubén came to work here. Trompi, you are my best friend and I cannot say anything you do not know. These years living with you (in León and in here) were good years! Regards also to your colleagues Annelies and Luca. A PhD student from Brazil came to their department in January 2011. Silvana, muchas gracias por todos los momentos que vivimos juntos estos años y por hacerme muy feliz. También por tu apoyo mientras preparaba mis exámenes. Espero que disfrutaras de nuestros días y viajes por Europa. Estoy seguro de que pronto nos volveremos a ver y, como tú dijiste, recuerda que es SIEMPRE!

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Miguelito

