University of Veterinary Medicine Hanover

Effects of dexmedetomidine and xylazine on cardiopulmonary function, recovery quality and duration and pharmacokinetics during total intravenous anaesthesia in horses

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by
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## Contents

1 Introduction ........................................................................................................ 13

2 Literature ........................................................................................................... 15

2.1 Drugs used for total intravenous anaesthesia ............................................ 15

2.1.1 Xylazine .............................................................................................. 15

2.1.2 Dexmedetomidine ............................................................................... 16

2.1.3 Midazolam.......................................................... .................................. 16

2.1.4 Ketamine.......................................................... .................................. 17

2.1.5 Total intravenous anaesthesia ............................................................ 18

2.2 Evaluation of degree of sedation .................................................................. 19

2.3 Assessment of anaesthetic quality ............................................................. 20

2.3.1 Cardiovascular function ....................................................................... 20

2.3.2 Respiratory function ............................................................................ 22

2.3.3 Anaesthetic depth ............................................................................... 23

2.3.4 Recovery quality and –time .................................................................. 24

2.4 Pharmacokinetic models ............................................................................ 25

2.4.1 Compartmental models of drug disposition after drug infusion ........... 26

2.4.1.1 One-Compartment Models........................................................... 26

2.4.1.2 Multicompartment Models .......................................................... 27

2.4.1.3 Noncompartment models ............................................................ 27

2.4.2 Recovery after intravenous infusion .................................................... 28

3 Materials and methods ...................................................................................... 30

3.1 Pretrials ...................................................................................................... 30

3.1.1 Animals ............................................................................................... 30

3.1.2 Study design ....................................................................................... 30

3.1.3 Instrumentation ................................................................................... 30
5.3.3 Instrumentation ................................................................................... 69
5.3.4 Drug combinations .............................................................................. 69
5.3.5 Experimental protocol ......................................................................... 70
5.3.6 Measurements .................................................................................... 71
5.3.7 Blood sampling .................................................................................... 71
5.3.8 Drug analysis ...................................................................................... 72
  5.3.8.1 Dexmedetomidine ........................................................................ 72
  5.3.8.2 Xylazine, ketamine and midazolam.............................................. 72
  5.3.8.3 Pharmacokinetic calculations....................................................... 72
5.3.9 Statistical analysis............................................................................... 73
5.4 Results ....................................................................................................... 73
  5.4.1 Anaesthesia ........................................................................................ 73
  5.4.2 Cardiorespiratory variables ................................................................. 74
  5.4.3 Pharmacokinetics............................................................................... 74
5.5 Discussion .................................................................................................. 74
5.6 Tables and Figures..................................................................................... 81
6 General discussion...................................................................................... 88
  6.1 Materials and methods .......................................................................... 88
    6.1.1 Study design .................................................................................... 88
    6.1.2 Preliminary trial ................................................................................ 88
    6.1.3 Anaesthetic protocol.......................................................................... 90
    6.1.4 Measurement methods ..................................................................... 90
    6.1.5 Blood sampling for pharmacokinetic analysis................................. 91
    6.1.6 Postoperative monitoring ................................................................. 92
  6.2 Results ....................................................................................................... 93
  6.3 Conclusion and outlook........................................................................... 99
7 Summary..................................................................................................... 100
Contents

8 Zusammenfassung ........................................................................................................ 103
9 Appendix .................................................................................................................... 106
10 Reference list ........................................................................................................... 117
List of abbreviations

° C degree Celsius
µg microgram
AV-blocks atrioventricular blocks
bwt body weight
c.v. coefficient of variation
CaO₂ arterial oxygen content
CC constant current
CI cardiac index
Cln confidence interval
cm centimetre
Cₘₐₓ peak plasma concentration
CNS central nervous system
CO cardiac output
CO₂ carbon dioxide
CRI constant rate infusion
CRT capillary refill time
D dexmedetomidine
DaO₂ oxygen delivery
DKM dexmedetomidine – ketamine – midazolam
dl decilitre
ECG electrocardiogram
EDTA ethylenediaminetetraacetate
E₇CO₂ endtidal carbon dioxide fraction
E₇O₂ endtidal oxygen fraction
FiO₂ fraction of inspired oxygen
g gram
<table>
<thead>
<tr>
<th>Abbreviation (Abbrev.)</th>
<th>Description</th>
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<tbody>
<tr>
<td>G</td>
<td>gauge</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
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<tr>
<td>Hb</td>
<td>haemoglobin</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromato graphy</td>
</tr>
<tr>
<td>HR</td>
<td>heart rate</td>
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<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>IM</td>
<td>intramuscular</td>
</tr>
<tr>
<td>IV</td>
<td>intravenous</td>
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<tr>
<td>$k_e$</td>
<td>elimination rate constant</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>kPa</td>
<td>kilopascal</td>
</tr>
<tr>
<td>kΩ</td>
<td>kiloohm</td>
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<tr>
<td>L</td>
<td>litre</td>
</tr>
<tr>
<td>LiCl</td>
<td>lithium chloride</td>
</tr>
<tr>
<td>mA</td>
<td>milliampere</td>
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<tr>
<td>MAP</td>
<td>mean arterial blood pressure</td>
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<tr>
<td>mg</td>
<td>milligram</td>
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<tr>
<td>min</td>
<td>minutes</td>
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<tr>
<td>ml</td>
<td>millilitre</td>
</tr>
<tr>
<td>mmHg</td>
<td>millimetre of mercury</td>
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<tr>
<td>mmol</td>
<td>millimol</td>
</tr>
<tr>
<td>ms</td>
<td>millisecond</td>
</tr>
<tr>
<td>ND</td>
<td>not done</td>
</tr>
<tr>
<td>NGD</td>
<td>nose to ground distance</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
</tr>
<tr>
<td>NWR</td>
<td>nociceptive withdrawal reflex</td>
</tr>
<tr>
<td>$O_2$</td>
<td>oxygen</td>
</tr>
<tr>
<td>OI</td>
<td>oxygenation index</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>PaCO₂</td>
<td>arterial partial pressure of carbon dioxide</td>
</tr>
<tr>
<td>PaO₂</td>
<td>arterial partial pressure of oxygen</td>
</tr>
<tr>
<td>PCV</td>
<td>packed cell volume</td>
</tr>
<tr>
<td>PR</td>
<td>pulse rate</td>
</tr>
<tr>
<td>PVR</td>
<td>peripheral vascular resistance</td>
</tr>
<tr>
<td>RR</td>
<td>respiratory rate</td>
</tr>
<tr>
<td>SaO₂</td>
<td>haemoglobin saturation</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>s</td>
<td>seconds</td>
</tr>
<tr>
<td>SV</td>
<td>stroke volume</td>
</tr>
<tr>
<td>SVI</td>
<td>stroke volume index</td>
</tr>
<tr>
<td>SVR</td>
<td>systemic vascular resistance</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>elimination half-time</td>
</tr>
<tr>
<td>TIVA</td>
<td>total intravenous anaesthesia</td>
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<tr>
<td>tₘₐₓ</td>
<td>time of maximal plasma drug concentration</td>
</tr>
<tr>
<td>TP</td>
<td>total protein</td>
</tr>
<tr>
<td>TTs</td>
<td>thermal thresholds</td>
</tr>
<tr>
<td>V</td>
<td>volt</td>
</tr>
<tr>
<td>V₃D</td>
<td>alveolar dead space fraction</td>
</tr>
<tr>
<td>WBC</td>
<td>white blood cell count</td>
</tr>
<tr>
<td>X</td>
<td>xylazine</td>
</tr>
<tr>
<td>XKM</td>
<td>xylazine – ketamine – midazolam</td>
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1 Introduction

Morbidity and mortality in equine anaesthesia is considerably greater than in other domestic species, which is mostly caused by hypotension and hypoventilation during anaesthesia (YOUNG et al. 1993). Currently prolonged general anaesthesia is accomplished by inhalation anaesthetics despite the fact that maintenance of anaesthesia with volatile agents carries a much higher risk of death (0.99%) than total intravenous anaesthetic maintenance (0.31%) (JOHNSTON et al. 2002; BIDWELL et al. 2007). The required concentrations of inhalation anaesthetics to provide a surgical plane of anaesthesia frequently contribute to intraoperative development of hypotension and hypoventilation (STEFFEY 2002).

In recent years, total intravenous anaesthesia (TIVA) protocols have become widely used in humans and also gained attention in equine anaesthesia research. The use of TIVA reduces the cost of equipment and eliminates any possible hazards to humans and environment associated with exposure to trace concentrations of volatile and gaseous anaesthetic drugs (SHINE 2010). The prevalent causes of death during equine anaesthesia are cardiac arrest, post-operative cardiovascular collapse, fractures and myopathies. Therefore, a stable cardiovascular function and an excellent recovery quality are important aims in developing new protocols for anaesthesia in horses.

A variety of techniques for TIVA have been investigated for surgical procedures up to 120 minutes (min) and more (YAMASHITA and MUIR 2009). Generally, anaesthesia is achieved with various combinations of α2-adrenoceptor agonists, dissociative anaesthetics, and centrally acting muscle relaxants. The use of guaifenesin, ketamine, and xylazine to induce and maintain anaesthesia in horses (“triple drip”) was first reported in 1978 (MUIR et al. 1978). These protocols produce minimum cardiovascular depression and moderate hypoventilation (GREENE et al. 1986). However, after an infusion period of 120 min the plasma concentrations of the active metabolite of ketamine, norketamine, as well as guaifenesin cumulate, reaching unacceptably high values (TAYLOR et al. 1995), leading to atactic and uncoordinated recoveries (MCCARTY et al. 1990).
The aims of the thesis were to determine whether a TIVA with a constant rate infusion of xylazine or dexmedetomidine, midazolam and ketamine can safely be used to maintain anaesthesia for 120 min in horses; whether the cardiopulmonary function and recovery period are dependent on the type (xylazine or dexmedetomidine) and dose of the $\alpha_2$-adrenergic agonist used; whether a reduced dose of ketamine in combination with the benzodiazepine midazolam provides sufficient depth of anaesthesia and whether the reversal of midazolam can improve the recovery quality. In addition, the elimination pharmacokinetics of ketamine, midazolam, xylazine and dexmedetomidine after two hours (h) of TIVA were determined.
2 Literature

2.1 Drugs used for total intravenous anaesthesia

All sedative and anaesthetic drugs influence cardiovascular function, but to a variable extent. To minimize adverse reactions, anaesthesia is commonly maintained with a combination of drugs to reduce the dose of single anaesthetics. Alpha<sub>2</sub>-adrenoceptor agonists produce sedation, anxiolysis, skeletal muscle relaxation and analgesia and are widely used for sedation, analgesia and premedication in horses (ENGLAND and CLARKE 1996). Commonly, α<sub>2</sub>-adrenoceptor agonists cause an initial hypertension followed by a prolonged hypotension together with a reduced cardiac output (CO) after bolus administration. They produce profound dose-related bradycardia, commonly accompanied by atrio-ventricular blocks (KERR et al. 1972; WAGNER et al. 1991; BETTSCHART-WOLFENSBERGER et al. 1999a; YAMASHITA et al. 2000; KÄSTNER et al. 2001; MURRELL and HELLEBREKERS 2005; KÄSTNER 2006). Premedication with α<sub>2</sub>-adrenoceptor agonists can reduce the release of catecholamines and therefore smooth both the induction and the maintenance of anaesthesia. In combination with ketamine, α<sub>2</sub>-adrenoceptor agonists are useful since they eliminate the muscular hypertonicity caused by the dissociative agent, whilst the decrease in heart rate (HR) and CO induced by the α<sub>2</sub>-adrenoceptor agonists are moderated by the sympathomimetic action of ketamine. The duration and intensity of cardiovascular depression depends on the type of α<sub>2</sub>-adrenoceptor agonist, its dose and route of administration (ENGLAND and CLARKE 1996).

2.1.1 Xylazine

Xylazine serves as the prototype and was the first α<sub>2</sub>-adrenoceptor agonist approved for the use in horses (CLARKE and TAYLOR 1986). Intravenous doses of xylazine used for equine chemical restraint range from 0.5 – 1.1 milligram/kilogram body weight, intravenously [mg/kg bwt IV] (MUIR 2009). Xylazine is less potent and has a lower α<sub>1</sub>:α<sub>2</sub>-selectivity than detomidine, romifidine or medetomidine (VIRTANEN et al. 1985; YAMASHITA et al. 2000). Cardiovascular depression is shorter and milder with xylazine than with equipotent doses of medetomidine and detomidine. Xylazine causes less initial hypertension with a minimal increase in peripheral vascular
resistance (YAMASHITA et al. 2000). The duration of cardiovascular depression seems to parallel the duration of the sedation (BRYANT et al. 1991; WAGNER et al. 1991). Reduction in arterial oxygen concentration and rise in arterial carbon dioxide concentration after xylazine are minimal (MUIR et al. 1979). At equisedative doses xylazine produces less ataxia of shorter duration compared to medetomidine (BRYANT et al. 1991). Xylazine has a short systemic half-life of 50 min after IV administration, similar to medetomidine (51.3 min) (GARCIA-VILLAR et al. 1981; BETTSCHART-WOLFENSBERGER et al. 1999b).

2.1.2 Dexmedetomidine
Medetomidine is a highly selective $\alpha_2$-adrenoceptor agonist and is a racemic mixture that contains equal parts of two optical enantiomers, dexmedetomidine and levomedetomidine (AANTAA et al. 1993). Dexmedetomidine is the sedative and analgesic active enantiomer (AANTAA et al. 1993). A dose of 3.5 $\mu$g/kg bwt IV of dexmedetomidine was equivalent to 7 $\mu$g/kg bwt IV of medetomidine and was successfully used for sedation in horses (BETTSCHART-WOLFENSBERGER et al. 2005). In dogs, analgesic effect of dexmedetomidine (20 $\mu$g/kg bwt, IV) lasted longer than the effect of the corresponding dose of racemic medetomidine (40 $\mu$g/kg bwt, IV) (KUUSELA et al. 2000). High doses of levomedetomidine enhanced bradycardia and reduced analgetic and sedative effects (KUUSELA et al. 2001). Cardiopulmonary side effects of dexmedetomidine were minimal. With dexmedetomidine, reduction in HR was not significant, and cardiac index (CI) was decreased only for the first ten minutes in horses. The initial increase with the following decrease in arterial blood pressure was of shorter duration than with medetomidine or xylazine (YAMASHITA et al. 2000; BETTSCHART-WOLFENSBERGER et al. 2005). Dexmedetomidine is the shortest acting $\alpha_2$-adrenoceptor agonist tested in horses with an elimination half-life of 19.80 ± 9.63 min in mature and 28.96 ± 7.61 min in geriatric ponies (BETTSCHART-WOLFENSBERGER et al. 2005).

2.1.3 Midazolam
Benzodiazepines, acting at the gamma-aminobutyric acid receptor, produce anxiolytic, muscle relaxant, and anticonvulsant effects and have the potential to
enhance the sedative-hypnotic effects of injectable and inhalant anaesthetics. Furthermore, midazolam causes anterograde amnesia (SALAND et al. 1992). Midazolam and other benzodiazepines produce little-to-no effect on cardiorespiratory variables in adult horses (KAEGI 1990; MUIR and MASON 1993; BETTSCHART-WOLFENSBERGER et al. 1996). In horses, midazolam has been used in conjunction with ketamine to induce general anaesthesia in a dose of 0.2 mg/kg bwt IV (LUNA et al. 1997). Midazolam has a fused imidazole ring which differentiates it from other benzodiazepines, e.g. diazepam. This structure is believed to account for the basicity, stability and rapid metabolism that distinguish midazolam from diazepam. The distribution half-life of this water-soluble benzodiazepine derivate is half that of diazepam and its total body clearance is much higher, giving midazolam a much shorter duration of action in humans (REVES et al. 1985). The incidence of thrombophlebitis and venous irritation is significantly less than that associated with diazepam (REVES et al. 1985). The effects of the benzodiazepines midazolam and climazolam can be reversed with the benzodiazepine antagonists flumazenil or sarmazenil (HOFFMAN 1993; BETTSCHART-WOLFENSBERGER et al. 1996; JOHNSON et al. 2003). To our knowledge, no data on disposition of midazolam and flumazenil in horses are available. In humans, elimination half-life ranges from 1.7 to 3.5 hours for midazolam and 40 to 80 min for flumazenil (ALLONEN et al. 1981; HEIZMANN et al. 1983; GREENBLATT et al. 1984; OLKKOLA and AHONEN 2008; MISAKA et al. 2010).

2.1.4 Ketamine
Ketamine as a dissociative anaesthetic decreases sensory input without blocking the brainstem or spinal pathways. Central nervous system (CNS) depression does occur in the thalamus and associated pain centres and minimally in the reticular formation, but subcortical areas and the hippocampus undergo activation (YAMASHITA and MUIR 2009). In contrast to other anaesthetics, ketamine increases HR, CO, arterial blood pressure and body temperature by CNS sympathetic activation (MUIR et al. 1999). The most common complications associated with the intravenous use of ketamine in horses are excitement or delirium during recovery including ataxia, dog-sitting or developing a brief period of severe muscle quivering and fasciculation
Cumulative drug effects are responsible for prolonged time of drug elimination and prolonged recovery periods of poor quality (YOUNG et al. 1993; BETTSCHART-WOLFENSBERGER et al. 1996; YAMASHITA et al. 2007). Elimination half-life after bolus administration of ketamine ranges from 42 to 66 min and elimination half-time after infusion of ketamine was 282 ± 32.6 min in horses (KAKA et al. 1979; WATERMAN et al. 1987; BETTSCHART-WOLFENSBERGER et al. 1996).

2.1.5 Total intravenous anaesthesia
The technique of TIVA for surgical anaesthesia using a mixture of guaifenesin, ketamine and xylazine has been successfully and routinely used in equine anaesthesia for several decades. YOUNG et al. (1993) used varying infusion rates of guaifenesin (100 - 110 mg/kg bwt/h), ketamine (2.0 - 2.8 mg/kg bwt/h) and xylazine (1.0 - 1.4 mg/kg bwt/h) for elective surgeries in horses. MUIR et al. (2000) reported for the same drug combination infusion rates of 75 mg/kg bwt/h of guaifenesin, 1.5 mg/kg bwt/h of ketamine and 0.75 mg/kg bwt/h of xylazine for surgical removal of abdominal testis in horses, but some horses needed higher infusion rates. A TIVA of guaifenesin, ketamine and detomidine was compared with halothane anaesthesia for surgical castration in ponies and it was shown that TIVA provided a much better cardiorespiratory function during anaesthesia (TAYLOR et al. 1998). In this study infusion rates for the first 60 min were 80 mg/kg bwt/h of guaifenesin, 3.2 mg/kg bwt/h of ketamine and 32 µg/kg bwt/h of detomidine which were reduced for the next 30 min to 60 mg/kg bwt/h, 2.4 mg/kg bwt/h and 24 µg/kg bwt/h (TAYLOR et al. 1998). BROCK and HILDEBRAND (1990) substituted successfully guaifenesin (100 mg/kg bwt) with diazepam (0.1 mg/kg bwt) in combination with ketamine for induction in horses. MCMURPHY et al. (2002) compared a TIVA containing guaifenesin (100 mg/kg bwt/h for 30min, then 50 mg/kg bwt/h for the next 45min), ketamine (6.6 mg/kg bwt/h) and romifidine (82.5 µg/kg bwt/h) with a halothane anaesthesia and showed that horses maintained higher arterial blood pressures during TIVA. KUSHIRO et al. (2005) reported that 25 mg/kg bwt/h guaifenesin could be substituted by 0.02 mg/kg bwt/h midazolam in horses. Recently, an infusion of a midazolam-ketamine-medetomidine combination [0.08 mg/kg bwt/h; 4 mg/kg bwt/h; 10 µg/kg bwt/h]
(YAMASHITA et al. 2007) and an infusion of a guaifenesin-ketamine-xylazine combination [131.1 mg/kg bwt/h; 5.6 mg/kg bwt/h; 1.4 mg/kg bwt/h] (BRINGEWATT 2009) was described for surgical anaesthesia for castration in horses.

Propofol is an anaesthetic suitable for prolonged TIVA (> 2 h) in horses and has some advantages over ketamine as it has a short context-sensitive half-life and does not produce active metabolites (NOLAN et al. 1996). In recent years propofol was often combined with medetomidine or with medetomidine and ketamine. These combinations were suitable to produce anaesthesia in ponies and horses for up to four hours with good and prompt recoveries. Thereby cardiovascular values were maintained within acceptable limits, but some horses developed hypoxia despite oxygen supplementation and spontaneous breathing (BETTSCHART-WOLFENSBERGER et al. 2001b, BETTSCHART-WOLFENSBERGER et al. 2001c) or positive pressure ventilation (BETTSCHART-WOLFENSBERGER et al. 2005, EDNER et al. 2002, OKU et al. 2005, UMAR et al. 2007). Nevertheless, propofol has some disadvantages as anaesthetic for horses that include high costs, large required volumes, poor analgesia and respiratory depression (MAMA et al. 1995, NOLAN and Hall 1985, YAMASHITA and MUIR 2009).

2.2 Evaluation of degree of sedation

To compare influences of different $\alpha_2$-adrenoceptor agonists on cardiopulmonary function and anaesthetic quality during TIVA, it is essential to elaborate a dosing regime that provides a comparable and consistent level of sedation. The extent and degree of head drop is a good indicator of sedation and can easily be determined without expensive equipment (KAMERLING et al. 1988; BRYANT et al. 1991; BETTSCHART-WOLFENSBERGER et al. 1999b). In addition to sedative effects of $\alpha_2$-adrenoceptor agonists analgesic effects are a main factor and qualitative parameter for anaesthesia. Noxious thermal stimulation is considered to be an objective and repeatable method for assessing cutaneous analgesia in horses (KAMERLING et al. 1985; KAMERLING et al. 1988; ROBERTSON et al. 2005).
2.3 Assessment of anaesthetic quality

Anaesthetic drugs exert profound effects on the cardiovascular system and cardiovascular function. All volatile anaesthetics cause dose-dependent cardiovascular depression and hypoventilation during anaesthesia (STEFFEY 2002), whereas cardiopulmonary parameters are maintained within acceptable limits with injectable drugs (BETTSCHART-WOLFENSBERGER et al. 2001b; BETTSCHART-WOLFENSBERGER et al. 2003; MAMA et al. 2005). Mean arterial blood pressure (MAP) of 60 mmHg or less during anaesthesia has been shown to increase the risk of postanaesthetic myopathy (GRANDY et al. 1987). But MAP may not be a good indicator of tissue perfusion as animals may have low CO with high systemic vascular resistance (SVR). Cardiac output measurements are a better indicator of cardiovascular function and organ perfusion (LINTON et al. 2000).

2.3.1 Cardiovascular function

Important components of cardiovascular function are CO, blood pressure, and oxygen delivery (DaO$_2$) to tissues. Cardiac output is a product of HR and stroke volume (SV). Heart rate, rhythm and cardiac murmurs can easily be determined by auscultation. The electrocardiography (ECG) is suited to determine in addition to HR and rhythm the electrical activity of the heart. Thus, ECG indicates if the myocardial perfusion and oxygenation is adequate or if electrolyte abnormalities exist. However, myocardial pump function or cardiac arrest can not be evaluated by the ECG, because normal ECG complexes can be monitored up to 20 min after euthanasia (YOUNG and TAYLOR 1993; SCHATZMANN 1995; YAMASHITA and MUIR 2009). Capillary refill time (CRT) and mucous membrane colour provide subjective information regarding haemoglobin concentration and oxygenation, peripheral vascular tone, and tissue perfusion. In addition to the ability to determine pulse rate and rhythm, palpation of the peripheral pulse provides a qualitative assessment of pulse pressure and is the difference between systolic and diastolic arterial blood pressure. It does not indicate perfusion pressure and should not be overestimated, because for example ketamine administration results in vasoconstriction and a reduction in pulse pressure even though perfusion pressure increases (MUIR et al. 1999; YAMASHITA and MUIR 2009). Arterial blood pressure is routinely measured.
during general anaesthesia in horses. Mean arterial blood pressures below 60 mmHg are associated with an increased incidence of complications, particularly development of postoperative rhabdomyolysis (GRANDY et al. 1987; LINDSAY et al. 1989; YOUNG and TAYLOR 1993). Arterial catheterization is the most reliable method for measuring arterial blood pressure and provides the possibility for sampling arterial blood gases (TAYLOR 1981). However, MAP is not a good indicator for tissue perfusion as animals may have low CO with high SVR. Cardiac output measurements allow assessment of the global cardiovascular function as major determinant for organ perfusion (LINTON et al. 2000). Knowledge of CO in anaesthetized horses can aid optimal titration of anaesthetic drugs and cardiovascular support. The CO is defined as the amount of blood pumped by the heart per min. A normal value for a resting adult horse is 32 - 40 litre/min (L/min). The CI, which is defined as CO divided by the body weight, of an adult horse is 72 – 88 ml/kg bwt/min (CORLEY et al. 2003). The CO can by determined by different validated methods. Doppler echocardiography as non-invasive ultrasound-based technique can be obtained with transesophageal or with transthoracic measurements and is not affected by intracardiac shunts. With the ultrasound beam being not parallel to blood flow, inaccuracies and increasing variability in measurements can occur. The correlation between transesophageal Doppler technique and thermodilution in anaesthetized horses was stronger than between transthoracic Doppler technique and thermodilution (YOUNG et al. 1996; LINTON et al. 2000). The background of indicator dilution methods for CO measurement is the indicator that is injected upstream of the heart into a vein and measured downstream, either in the pulmonary artery or a peripheral artery. The greater the CO, the greater the effective dilution of the indicator and therefore the smaller the area under the time-concentration curve (CORLEY et al. 2003). Indicator dilution techniques with indocyanine green, cold (thermodilution) and lithium as marker have been used in the horse. Indocyanine green measurement requires extensive calibration of the densitometer with samples of the individual patient’s blood and large volumes of withdrawn blood (MIZUNO et al. 1994). Thermodilution has been extensively used in equine research, in adults and in foals. The advantages are the absence of
accumulation of the indicator, the inexpensive indicator and the possibility for multiple repeated measurements. A disadvantage is the need for catheterization of the right heart (CORLEY et al. 2003). The lithium dilution technique offers the advantage, that only peripheral venous catheters are necessary. There is a good agreement between lithium dilution and thermodilution in anaesthetized horses (LINTON et al. 2000) and in foals (CORLEY et al. 2002). However, the accuracy of lithium dilution may decrease with a large number of repeated measurements because of lithium accumulation. The pulse contour analysis offers the possibility to determine CO continuously throughout arterial pressure waveform. The area under the arterial pressure time curve during systole represents the blood flow through the catheterized vessel and therefore reflects CO (GREVES et al. 1968). The beginning of the systole is marked by the initial rapid increase of the pressure curve, and the end is marked by the dicrotic notch (CORLEY et al. 2003). The blood flow and corresponding pressure curve is dependent on the elasticity of arteries and varies with age, gender, and subject size, which makes an individual calibration for each patient necessary (CORLEY et al. 2003). The algorithms used for calculation of CO are derived from humans and hamper the use of this technique for horses. In horses, the accuracy and correlation of the pulse contour analysis with the lithium dilution technique is largely dependent on the recalibration interval, which should not exceed 20 up to 30 min. Longer intervals result in significant deviation between lithium dilution technique and pulse contour analysis that is further influenced by inotropic / vasoactive drugs (CORLEY et al. 2003; HALLOWELL u. CORLEY 2005; SCHAUVLIEGE et al. 2009).

2.3.2 Respiratory function

The respiratory function in horses is altered by anaesthetic drugs and effects of body position during recumbency (HALL et al. 1968; STEFFEY et al. 1977b; GLEED and DOBSON 1988). The ventilation and pulmonary gas exchange is monitored to ensure adequate oxygen (O₂) and carbon dioxide (CO₂) exchange and oxygenation of haemoglobin. The respiratory rate can easily be obtained by chest wall movement or changes of volume of the rebreathing bag. While hypoventilation or apnoe are identified directly, pulmonary gas exchange, especially hypoxia or hypercapnia, can not be assessed exactly (TAYLOR and CLARK 2007; YAMASHITA and MUIR 2009).
Capnometry and pulse oxymetry are two non-invasive methods to analyse exhaled carbon dioxide tension and percentage of oxygenated arterial blood haemoglobin. The $CO_2$ tension within the endotracheal tube at the end of expiration ($E_{T}CO_2$) should theoretically equal the $CO_2$ tension in the arterial blood ($PaCO_2$) leaving the alveoli. The $E_{T}CO_2$ and $PaCO_2$ correlate positively but in horses $E_{T}CO_2$ tends to be 10 to 15 mmHg lower than $PaCO_2$ (CRIBB 1988; YAMASHITA and MUIR 2009). The $E_{T}CO_2$ provides a relatively poor indication of $PaCO_2$ in both, healthy and compromised horses, especially during spontaneous ventilation and after anaesthesia longer than 60 min and should never substitute blood gas analysis (GEISER and ROHRBACH 1992; KOENIG et al. 2003). Analysis of arterial blood is an exact method to determine blood haemoglobin concentration, arterial pH, arterial partial pressure of oxygen ($PaO_2$) and $PaCO_2$, as well as haemoglobin saturation. The $PaCO_2$ is used to assess hypo- or hyperventilation as it reflects the balance between metabolic production of carbon dioxide and its elimination by the lungs. The $PaO_2$ is used to determine if arterial blood oxygenation is adequate. A reduction of $PaO_2$ is caused by diffusion abnormalities, ventilation-perfusion mismatching or right to left shunts, whereas $PaCO_2$ is rarely elevated under these conditions because of the stimulation of ventilation as a result of hypoxaemia (HUBBELL and MUIR 2009). The alveolar dead space fraction ($V_D$) can be used to determine the amount of wasted ventilation and is in our study, derived from standard formulas (Robinson 2009).

2.3.3 Anaesthetic depth
Unconsciousness is a mainstay of general anaesthesia. Horses with insufficient anaesthetic depth may respond to surgical stimulation, resulting in stress, movement and danger for the horse itself and the surrounding environment. Horses that are anaesthetized to deeply may suffer excessive cardiorespiratory depression, resulting in a poor and prolonged recovery and myopathy. Clinically anaesthetic depth can be assessed by physical signs, including movement, the position of the eye, the degree of depression of the protective reflexes of the eye, the loss of the swallowing reflex, the rate and depth of breathing, and the horse’s response to surgical stimulation. The use of dissociative anaesthetics constricts the use of these physical signs for anaesthetic depth assessment. Some horses develop an apneusitic pattern of
breathing and reduced minute volume. Pharyngeal and laryngeal reflexes remain active after ketamine administration. Lacrimation and ocular and palpebral reflexes are more pronounced in horses administered ketamine (YAMASHITA and MUIR 2009).

An experimental method allowing comparison of anaesthetic drugs requires a standardized repeatable nociceptive stimulation. In horses, a square-shaped constant voltage electrical stimulation at 50 volts (V) and 5 hertz (Hz), applied through needle electrodes inserted deeply in the gingiva, is commonly used for minimum alveolar concentration (MAC) determination (STEFFEY et al. 1977a; PASCOE et al. 1993; BENNETT et al. 2004). Surface electrodes have also been used over the distal digital nerve (DOHERTY et al. 1997). Constant current (CC) stimulation has been used to improve reproducibility and sensitivity in nerve stimulation studies in horses (SPADAVECCHIA et al. 2002). For MAC determination in ponies CC surface electrode stimulations were more repeatable than using constant voltage on the gingival needle- or surface-electrodes (LEVIONNOIS et al. 2009). The nociceptive withdrawal reflex (NWR) threshold was significantly higher and latency was significantly longer for the hind limb than for the forelimb. This was associated with significantly stronger behavioural reactions at forelimbs (SPADAVECCHIA et al. 2003).

2.3.4 Recovery quality and –time
For equine anaesthesia, 25 - 50 % of fatalities are a direct result of injury sustained during recovery (YOUNG u. TAYLOR 1993; DONALDSON et al. 2000; JOHNSTON et al. 2002; BIDWELL et al. 2007). In addition to the horse’s physical condition and temperament, the environment at the recovery site and the type of surgery the quality and time of recovery is related to the dose and route of anaesthetic drug administration, the duration of anaesthesia, the cardiopulmonary function during maintenance of anaesthesia and the administration of sedatives or drug antagonists during the recovery period (YOUNG and TAYLOR 1993; JOHNSTON et al. 2002). A challenge for the anaesthetist is to find a dosing regime that provides adequate anaesthetic depth and ensures that horses regain sufficient strength and coordination.
before they try to stand up. Especially for injectable drugs used for prolonged anaesthesia cumulative drug effects and prolonged time for drug elimination lead to prolonged recoveries and horses being atactic and tense during recovery period (MCCARTY et al. 1990; YOUNG et al. 1993; TAYLOR et al. 1995; BETTSCHART-WOLFENSBERGER et al. 1996; YAMASHITA et al. 2007). Assessment of the recovery phase is an important parameter to judge the quality of different anaesthetic protocols. The recovery scoring systems should objectively evaluate recovery quality with a high reproducibility and repeatability. A number of scoring systems to evaluate recovery quality have been developed, but with different emphases of features that are directly linked to injury (MATTHEWS et al. 1998; DONALDSON et al. 2000; RAY-MILLER et al. 2006; CLARK-PRICE et al. 2008; SUTHERS et al. 2011). As objective evaluation of recovery quality is challenging, it should be multifactorial and contain aspects as coordination of movements, the number of attempts to sternal and standing and overall quality of recovery and strengthen categories that are directly associated with increasing risk of injury during recovery (CLARK-PRICE et al. 2008). Additionally, separate evaluation of time to get nystagmus, to first movement of ears, head and legs, to first attempt to reach sternal and standing as well as to take sternal and standing position is helpful to quantify and compare recovery quality of different anaesthetic protocols.

2.4 Pharmacokinetic models
A major challenge regarding the use of TIVA in horses is the long-acting and cumulative character of intravenous anaesthetics that may prolong and worsen recovery and impair the maintenance of adequate anaesthetic depth for a longer period. For volatile anaesthetics, the end-tidal concentration of inhaled anaesthetic drugs can be monitored easily to determine the dose-response effects that ensure adequate anaesthetic depth. The end-tidal partial pressure of the anaesthetic gas is an index of the partial pressure of the gas in the alveoli and brain and serves as a measure of the quantity of anaesthetic being delivered. Measurement of alveolar concentrations enables determination of anaesthetic depth with a low error rate (LEVIONNOIS 2007; YAMASHITA and MUIR 2009). For TIVA, concentration of anaesthetic drugs in the CNS is a result of a complex of distribution, metabolism and
elimination (LEVIONNOIS 2007). Measurement of plasma concentration is more difficult and can not be performed directly during anaesthesia. Therefore, knowledge of the pharmacokinetic properties of anaesthetics is important to produce homogenous plasma concentration over a longer infusion period. Cumulative drug effects are a main reason to avoid TIVA including ketamine and guaifenesin for longer procedures. Total intravenous anaesthesia mostly consists of a combination of two, or more often three drugs (see above), that have different pharmacokinetic properties. To dose drugs individually with respect to the different pharmacokinetic properties, the use of different syringe pumps for application of the drugs is beneficial. The continuous variable rate infusion of intravenous anaesthetics provides a practicable and controllable method that produces more homogenous plasma concentrations and effects than the application of incremental intravenous bolus, which leads to cyclical fluctuations in plasma concentration, anaesthetic depth, and cardiorespiratory function (ROBINSON 2009).

2.4.1 Compartmental models of drug disposition after drug infusion

Modelling of drug disposition is a mathematical tool to predict changes of plasma concentration over time. Each drug and each horse requires its own model as it fits to observed drug behaviour. Nevertheless, it is important to realize, that the effect compartment representing the CNS is not included in modelling although there is a half-life for equilibration resulting in a time-lag before concentration changes in plasma are reflected in the effect compartment (HILL 2004).

2.4.1.1 One-Compartment Models

The one-compartment model is the simplest model of drug disposition but is rarely used for drugs used for anaesthesia (SAMS and Muir 2009). Plasma concentration in the one compartment is dependant on the infusion rate of the drug and the rate of excretion. As the infusion is started, the plasma concentration increases in a negative exponential fashion. The concentration at equilibrium is determined by the ratio of infusion rate to the clearance rate of the drug. After stopping the infusion, the plasma concentration declines following a simple single exponential curve whereat the time constant will be that of excretion. The elimination half-life will be the same for a
constant infusion with or without reaching equilibrium. Thus, the time for halving the plasma concentration will always be the same, independent of the duration of infusion (HILL 2004).

2.4.1.2 Multicompartment Models
Multicompartment models consist of one central compartment which represents the changes in plasma drug concentration and peripheral compartments. These compartments model regions of the body that can temporarily receive amounts of drug from the central compartment, and later release them back into the plasma (SAMS and MUIR 2009). Thereby, a drug can only enter and leave the model system through the central compartment (DISTEFANO and LANDAW 1984). After starting a fixed infusion rate, the plasma concentration of the central compartment will increase rapidly, but distribution to the peripheral compartments and excretion will remove drug from the plasma. The movement of the drug is dependent on the concentration gradients between the plasma and peripheral compartments. This distribution is reversible as the drug must return to the central compartment for elimination. A steady-state is reached when there is no inter-compartmental movement of the drug and input to the central compartment from infusion is the same as output from excretion (HILL 2004). The decline of the plasma concentration is dependent on the duration of infusion. After a short infusion period, plasma concentration will halve themselves in a very short time because of the combined effects of distribution to peripheral compartments and excretion. With increasing time of infusion, the concentration gradients between central and peripheral compartments are lower, so the contribution of distribution is minimized and elimination occurs only by excretion and is opposed by redistribution from peripheral to central compartments. The longest time to halve plasma concentration occurs after equilibrium is achieved (HILL 2004; SAMS and MUIR 2009).

2.4.1.3 Noncompartment models
Noncompartmental analysis uses the techniques derived from statistical moment theory (BOVILL 2005) and is based on the theory that the movement of drug molecules within the body is considered to be a series of random events (MARTINEZ
1998). In contrast, the compartment models requires certain assumptions about the system's behaviour (MARTINEZ 1998). The basis noncompartment model comprises a central measurement pool with a sum of all de novo entry of the substances into it and the sum of substances that are irreversibly removed by metabolism, degradation or excretion. Recirculation or exchanges to the central pool can occur with any number of noncentral pools, but none of which has to be identified with any physiological structures (DISTEFANO and LANDAW 1984). With compartment models, data for most individuals may be consistent with one type of model, but some may be consistent with a different type of model. These inconsistencies lead to criticism of the compartment models (BONATE 2005). A further advantage of using the noncompartment model is that no assumptions are required how a drug partitions across body tissues (MARTINEZ 1998). For compartment data analysis the risk for model misspecification can lead to clinically important faults in data interpretation. Model misspecification can occur especially with a highly variable dataset or for studies with a poorly designed sampling schedule (MARTINEZ 1998).

### 2.4.2 Recovery after intravenous infusion

Plasma concentrations for an intravenous infusion are influenced by distribution, redistribution, metabolism and excretion. Traditionally, elimination characteristics are described by the elimination half-life of a drug. This means the time required for one half of the drug to be removed from the body after a single bolus administration and is determined by the volume of distribution and clearance (SAMS and MUIR 2009). A newer method to describe elimination pharmacokinetics after a continuous drug infusion is the context-sensitive half-life. This is the time required for the plasma drug concentration to decline by 50% after termination of an infusion and is determined by the rate at which the drug is irreversibly removed from the body, as well as the rate of redistribution to peripheral tissues. The context-sensitive half-time has been proposed as a more useful measure of the pharmacokinetic offset of intravenous anaesthetics (HUGHES et al. 1992; COETZEE 2005). FISHER and ROSEN (1986) found out that the return of neuromuscular function after administration of muscle relaxants after a variety of dosing schemes were not predicted by the elimination half-life, but were dependent on the duration of administration. KAPILA et al. (1995)
evaluated the accuracy of the elimination half-life of alfentanil and remifentanil to
describe a 50 % decrease of plasma concentration after a three hour infusion time
and compared this with a calculated and measured context-sensitive half-time. While
the 50 % decrease in drug concentration was successfully predicted by context-
sensitive half-time, it was not by the elimination half-life. Even after an infusion
duration far longer than necessary to achieve a steady-state, which implicates an
equilibration of drug concentration within plasma and peripheral compartments, the
measured context-sensitive half-time deviates from terminal elimination half-life
(KAPILA et al. 1995).
3 Materials and methods

3.1 Pretrials

<table>
<thead>
<tr>
<th>3.1.1 Animals</th>
<th>manuscript I page 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.2 Study design</td>
<td>manuscript I page 35</td>
</tr>
<tr>
<td>3.1.3 Instrumentation</td>
<td>manuscript I page 35</td>
</tr>
<tr>
<td>3.1.4 Thermal threshold testing</td>
<td>manuscript I page 36</td>
</tr>
<tr>
<td>3.1.5 Determination of head height</td>
<td>manuscript I page 36</td>
</tr>
<tr>
<td>3.1.6 Treatment groups</td>
<td>manuscript I page 36</td>
</tr>
</tbody>
</table>
### 3.2 Total intravenous anaesthesia (main trials)

<table>
<thead>
<tr>
<th>Section</th>
<th>Manuscript I Page</th>
<th>Manuscript II Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.1 Animals</td>
<td>37</td>
<td>67</td>
</tr>
<tr>
<td>3.2.2 Study design</td>
<td>37</td>
<td>68</td>
</tr>
<tr>
<td>3.2.3 Drug combinations</td>
<td>37</td>
<td>68</td>
</tr>
<tr>
<td>3.2.4 Instrumentation</td>
<td>38</td>
<td>68</td>
</tr>
<tr>
<td>3.2.5 Cardiac output determination</td>
<td>39</td>
<td>70</td>
</tr>
<tr>
<td>3.2.6 Electrical stimulation</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>3.2.7 Experimental protocol</td>
<td>40</td>
<td>69</td>
</tr>
<tr>
<td>3.2.8 Recovery assessment</td>
<td></td>
<td>114</td>
</tr>
<tr>
<td>3.2.9 Cardiopulmonary measurements</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>3.2.10 Blood sampling</td>
<td></td>
<td>70</td>
</tr>
<tr>
<td>3.2.11 Drug analysis</td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>3.2.12 Pharmacokinetic calculations</td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>3.2.13 Statistical analysis</td>
<td>42</td>
<td>72</td>
</tr>
</tbody>
</table>
4 Manuscript I

Effects of dexmedetomidine and xylazine on cardiovascular function and recovery quality and duration during total intravenous anaesthesia in horses

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4.1 Abstract

Objectives: To compare cardiovascular effects and the recovery quality and duration of total intravenous anaesthesia (TIVA) with xylazine-ketamine-midazolam (XKM) or dexmedetomidine-ketamine-midazolam at two different doses (DKM1 and DKM2).

Methods: Prospective, randomized experimental cross-over trial. In pretrials with five horses equisedative doses of xylazine (X) and dexmedetomidine (D) for constant rate infusion were determined by nose to ground distance (NGD) and thermal stimulation. Eight horses were anaesthetized three times for two hours. After acepromazine (0.03 mg/kg bwt, IM), xylazine (0.5 mg/kg bwt, IV) or dexmedetomidine (3.5 µg/kg bwt, IV) anaesthesia was induced with ketamine (2.5 mg/kg bwt, IV) and midazolam (0.05 mg/kg bwt, IV). The TIVA was maintained with xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1] or dexmedetomidine (7 µg/kg bwt/h)
Manuscript I

[DKM2] in combination with midazolam (0.1 mg/kg bwt/h) and ketamine (3 mg/kg bwt/h). Ketamine was increased in response to positive reactions to electrical nociceptive stimulation performed every 30 min. Arterial blood gases, heart rate, mean arterial blood pressure, and cardiac index [LiDCOplus-monitor] were measured before treatment (baseline), after sedation, and during anaesthesia. Twenty minutes after the end of TIVA, flumazenil (0.01 mg/kg bwt, IV) was administered. Recovery quality and duration were assessed. A two-way analysis of variance with repeated measurements was used to analyse data with an alpha of 5%.

**Results:** Dexmedetomidine (3.5 µg/kg bwt, IV [bolus] and 7 µg/kg bwt/h [CRI]) was equisedative to xylazine (0.5 mg/kg bwt, IV [bolus] and 1 mg/kg bwt/h [CRI]) based on NGD and thermal stimulation. During TIVA MAP was not significantly different from baseline and HR decreased significantly from baseline in all three groups. With XKM CI was not considerably different from baseline at all time points, but had a trend to increase continuously reaching higher values than baseline (88.3 ± 39.2 ml/kg bwt) at 120 min (114.9 ± 30.0 ml/kg) [p=0.68]. With DKM1 and DKM2 CI decreased significantly from baseline (85.6 ± 37.5 ml/kg bwt and 86.0 ± 26.1 ml/kg bwt) to sedation values (65.3 ± 28.9 ml/kg bwt and 62.4 ± 16.4 ml/kg bwt) and remained low with DKM2 during the first h of TIVA (61.0 ± 17.7 ml/kg bwt and 64.4 ± 15.6 ml/kg bwt). During TIVA CI was significantly higher with XKM than with DKM2. Mean ketamine doses of 3.7 mg/kg bwt/h (XKM and DKM2) and 3.6 mg/kg bwt/h (DKM1) were required. Recovery quality was good to excellent with a mean duration of 37.1 ± 16.1, 31.1 ± 8.9 and 45.5 ± 20.9 min with XKM, DKM1 and DKM2, respectively, resulting in a significant difference between DKM1 and DKM2.

**Conclusion:** All three drug combinations are suitable to maintain anaesthesia for two hours with good to excellent recovery conditions. The dexmedetomidine dose being equisedative to xylazine in conscious horses induced more cardiovascular depression than xylazine during TIVA.

**Key words:** equine, xylazine, dexmedetomidine, ketamine, midazolam, flumazenil, total intravenous anaesthesia.
4.2 Introduction

Morbidity and mortality in equine anaesthesia is considerably higher than in other domestic species, which is often related to hypotension and hypoventilation during anaesthesia (YOUNG et al. 1993). The maintenance of anaesthesia with volatile agents seems to carry a much higher risk of death than TIVA (JOHNSTON et al. 2002; BIDWELL et al. 2007). All volatile anaesthetics cause dose-dependent cardiopulmonary depression and hypoventilation during anaesthesia (STEFFEY 2002), whereas cardiopulmonary parameters are maintained better during infusion of injectable drugs (BETTSCHART-WOLFENSBERGER et al. 2001a; BETTSCHART-WOLFENSBERGER et al. 2003; MAMA et al. 2005). In addition, TIVA does not require expensive equipment for its administration and eliminates any possible hazards to humans associated with exposure to trace concentrations of volatile and gaseous anaesthetic drugs (ROBINSON 2009).

A variety of ketamine based TIVA techniques have been investigated from short (< 30 min) up to prolonged procedures with 120 min and more (ROBINSON 2009). The combination of guaifenesin, ketamine and α2-adrenoceptor agonists (xylazine, detomidine, romifidine) is a common combination for TIVA in the horse known as “triple drip” (MCCARTY et al. 1990; TAYLOR et al. 1995; MCMURPHY et al. 2002). The routine use of this type of TIVA for prolonged periods of anaesthesia is hampered by cumulative drug effects and prolonged time for drug elimination, which leads to prolonged recoveries with poor quality (YOUNG et al. 1993; BETTSCHART-WOLFENSBERGER et al. 1996; YAMASHITA et al. 2007). Pharmacokinetic studies showed, that plasma concentrations of norketamine, the active metabolite of ketamine, increased progressively and also guaifenesin concentrations increased, reaching unacceptably high values after two hours of anaesthesia (TAYLOR et al. 1995). This may lead to horses being tense and atactic during the recovery period (MCCARTY et al. 1990). Guaifenesin concentrations increased continuously during TIVA with ketamine, guaifenesin and detomidine (TAYLOR et al. 1995; LUNA et al. 1996). Guaifenesin has volume and concentration limitations, because solutions more concentrated than 15 % are difficult to keep in solution, and they cause intravascular haemolysis and aseptic thrombophlebitis (SCHATZMANN et al. 1978;
GRANDY and MCDONELL 1980; HERSCHL et al. 1992). To improve recovery quality, benzodiazepines including diazepam (ROSSETTI et al. 2008), climazolam (BETTSCHART-WOLFENSBERGER et al. 1996) and midazolam (YAMASHITA et al. 2007) have been used instead of guaifenesin to provide muscle relaxation because of the possibility to antagonize their effects with flumazenil or sarazenil.

During TIVA CNS depression and analgesia are often obtained by \( \alpha_2 \)-adrenoceptor agonists, including xylazine (MCCARTY et al. 1990; SINCLAIR and VALVERDE 2009), detomidine (TAYLOR et al. 1995; LUNA et al. 1996), romifidine (MCMURPHY et al. 2002; ROSSETTI et al. 2008) or medetomidine (BETTSCHART-WOLFENSBERGER et al. 2001b; BETTSCHART-WOLFENSBERGER et al. 2001c; BETTSCHART-WOLFENSBERGER et al. 2003; UMAR et al. 2007; YAMASHITA et al. 2007). They all cause cardiovascular depression with a significant decrease in cardiac output (CO). The duration and intensity of this effect depends on the type of \( \alpha_2 \)-adrenoceptor agonist, its dose and route of administration (ENGLAND and CLARKE 1996). Dexmedetomidine, the active D-enantiomer of medetomidine, is a highly selective \( \alpha_2 \)-adrenoceptor agonist (VIRTANEN et al. 1988; VICKERY and MAZE 1989) and twice as potent as the racemic mixture of medetomidine (VIRTANEN et al. 1988). Dexmedetomidine causes similar cardiopulmonary changes to other \( \alpha_2 \)-adrenoceptor agonists, but of very short duration and the pharmacokinetic profile makes it suitable for prolonged infusion (BETTSCHART-WOLFENSBERGER et al. 2005). After a single bolus injection xylazine also has a short duration of action and elimination half-life (KERR et al. 1972; GARCIA-VILLAR et al. 1981; BRYANT et al. 1991; YAMASHITA et al. 2000). The objective of this study was to compare three different TIVA protocols based on midazolam and ketamine combined with xylazine or dexmedetomidine at a high or low dose and subsequent reversal of midazolam with flumazenil. Cardiopulmonary effects, anaesthetic quality and recovery quality and time were evaluated.
4.3 Materials and Methods

4.3.1 Pretrials

4.3.1.1 Animals

Five adult, healthy university-owned horses (three warmbloods, two standardbreds), two geldings and three mares, weighing 540 ± 52.1 kg (mean ± standard deviation [SD]) [487 to 600 kg] and aged 12 ± 7.9 years [5 to 23 years] were used. The experimental protocol was approved by the Ethical Committee of the Lower Saxony State Office for Consumer Protection and Food Safety.

4.3.1.2 Study design

The study was carried out as a prospective, randomized experimental cross-over trial. Each horse was tested three times with at least four weeks between trials.

4.3.1.3 Instrumentation

Two hours before each trial the skin over the right and left jugular vein was clipped and surgically prepared. After local block with mepivacaine hydrochloride (Scandicain® 2%, AstraZeneca GmbH, Wedal, Germany), a 13 gauge (G) catheter (Vygonüle S, Vygon GmbH & Co. KG, Aachen, Germany) was placed in each jugular vein. One catheter was used for blood sampling, the other for drug administration and was connected to extension tubes (Lectro-cath, Laboratoires Pharmaceutiques VYGON, Ecouen, France). Heart rate was measured by a base apex lead electrocardiogram (Televet 100, Fa. Kruuse, Marslev, Denmark).

Thermal thresholds (TTs) were measured by a remote controlled TT testing device adapted to the use in horses [Wireless Thermal Threshold Testing System WTT2, Topcat Metrology Ltd, Gravel Head Farm, Little Downham]. The probe with a heater element and adjacent temperature sensor was placed above the right nostril and held in place with an elastic strap. Consistent contact between the probe and the skin was ensured by inflating a blood pressure bladder to a constant pressure between 30 and 80 mmHg. The probe was connected to the control unit which was placed over the
horse’s withers. The rate of heating was 0.8 °C/s with a cut-out set at 60 °C to prevent thermal burns.

### 4.3.1.4 Thermal threshold testing

Before each test, the skin temperature was recorded. The thermal stimulus was applied via a handheld infrared remote control from an operator standing behind the horse. When a positive reaction was observed, heating was terminated and the threshold recorded. A positive reaction was defined as rubbing the nose against the wall or the foreleg, shaking of the head, twitching the nose, turning the head towards the operator or pinning back the ears.

Before any treatments were given, four baseline pre-treatment TTs were recorded at 15 min intervals, then treatment was given at time zero. The thermal thresholds were determined 15, 30, 60, 90 and 120 min after starting the infusion of the tested α2-adrenoceptor agonists as well as 30 and 60 min after stopping the infusion.

### 4.3.1.5 Determination of head height

The horses were placed in stocks with a scale on the wall next to the horses head. Movement of the head was not restricted. In five minutes intervals HR, respiratory rate (RR) and the distance between ground and the rostral part of the nostrils (NGD) were recorded, starting directly after bolus of the α2-adrenoceptor agonist was given. For baseline determination the animals were observed for one hour. The mean these measurements were defined as baseline. The baseline NGD was set as 100 %. At the end the time to regain normal head position was noted and the percentage of reduction of the NGD compared to baseline was calculated.

### 4.3.1.6 Treatment groups

For group X, horses were treated with xylazine (Xylazine® 2%, CP-pharma GmbH, Burgdorf, Germany). A bolus of 0.5 mg/kg bwt IV was administered over a period of two minutes followed by a CRI of 1 mg/kg bwt/h xylazine IV over 120 min.
For group D1, horses were treated with an initial bolus of 3.5 µg/kg bwt dexmedetomidine IV (Dexdomitor® 0.5 mg/ml, Orion-Farmos, Turku, Finland) followed by a CRI of 5 µg/kg bwt/h.

Since the tested dexmedetomidine dose did not achieve the same NGD as the xylazine protocol, a third trial was added (group D2). The percentage of NGD reduction in each horse after xylazine was transferred to the current baseline NGD and defined as threshold. Horses were treated with a bolus of 3.5 µg/kg bwt IV dexmedetomidine followed by a CRI of 5 µg/kg bwt/h IV. If the nostril position was higher than the previously defined threshold with a range of five per cent, a 0.5 mg bolus of dexmedetomidine was injected. The number of required injections and the total dexmedetomidine dose over the infusion period were calculated.

4.3.2 Total intravenous anaesthesia

4.3.2.1 Animals

Eight adult university-owned horses, two geldings and six mares, weighing 525 kg ± 54.4 kg (mean ± SD) [450 to 580 kg] and aged 13.5 ± 6.8 years [5 to 23 years] were used. The study group consisted of four standardbreds, one thoroughbred and three warmblood horses. All horses were considered to be healthy by clinical and laboratory examination and echocardiography. Food, but not water, was withheld six hours before anaesthesia. The experimental protocol was approved by the Ethical Committee of the Lower Saxony State Office for Consumer Protection and Food Safety.

4.3.2.2 Study design

The study was carried out as a prospective, randomized experimental cross-over trial. Each horse was anaesthetized three times with at least four weeks between anaesthetic episodes.

4.3.2.3 Drug combinations

All horses were premedicated with acepromazine (0.03 mg/kg bwt IM) [Vetranquil® 1%, Albrecht GmbH, Aulendorf, Germany] 15 min before sedation with the tested α2-
adrenoceptor agonist. Three drug combinations were used for TIVA. Combination 1 (XKM) consisted of sedation with 0.5 mg/kg bwt xylazine IV followed by ketamine (2.2 mg/kg bwt, IV) [Narketan® 100mg/ml, Vétoquinol GmbH, Ravensburg, Germany] and midazolam (0.05 mg/kg, bwt IV) [Midazolam-ratiopharm® 15mg/3ml, ratiopharm GmbH, Ulm, Germany] for induction of anaesthesia. Anaesthesia was maintained by infusion of ketamine (starting dose of 3 mg/kg bwt/h, IV) midazolam (0.1 mg/kg bwt/h, IV) and xylazine (1.0 mg/kg bwt/h, IV). Combination 2 and 3 (DKM1 and DKM2) consisted of IV administration of 3.5 µg/kg bwt dexmedetomidine for sedation followed by ketamine (2.2 mg/kg bwt, IV) and midazolam (0.05 mg/kg bwt, IV) for induction of anaesthesia. Anaesthesia was maintained with ketamine (starting dose of 3 mg/kg bwt/h, IV), midazolam (0.1 mg/kg bwt/h, IV) and dexmedetomidine at a dose of 5 µg/kg bwt/h IV or 7 (µg/kg bwt/h, IV) in group DKM1 or DKM2, respectively.

4.3.2.4 Instrumentation

Before anaesthesia, each horse was weighed and body temperature, HR, and RR were obtained. Packed cell volume (PCV), white blood cell count (WBC), total protein (TP), haemoglobin concentration (Hb) and plasma sodium concentrations (Sysmex® KX-21, Sysmex Deutschland GmbH, Norderstedt, Germany and Vitros® System, DTE II module, Ortho-Clinical Diagnostics GmbH, Neckargemünd, Germany) were determined from venous blood. The skin over the right and left jugular vein was clipped and surgically prepared. After infiltration of the skin with mepivacaine hydrochloride (Scandicain® 2%, AstraZeneca GmbH, Wedal, Germany), a 12 G catheter (EquiCath™ Fastflow, Braun, Tuttlingen, Germany) was placed into each jugular vein, one close to the superior thoracic aperture for drug administration and one for blood collection. The skin over the right transverse facial artery was shaved, cleaned and desensitized with a transdermal anaesthetic cream containing lidocaine and prilocaine (EMLA®, AstraZenca GmbH, Wedel, Germany). For direct arterial blood pressure measurement and cardiac output determination, a 20 G catheter (Venocan™ IV Catheter, Kruuse, Langeskov, Denmark) was placed in the right transverse facial artery and connected to a pre-calibrated disposable pressure transducer (DTXPlus® Pressure Transducer System, Becton Dickinson GmbH, Heidelberg, Germany), set at the level of the right atrium. Pressure readings were
displayed and recorded with a commercial anaesthesia monitoring machine (Cardiocap™/5, Datex-Ohmeda GmbH, Duisburg, Germany).

### 4.3.2.5 Cardiac output determination

Cardiac output measurements were performed by lithium dilution and pulse contour analysis [LiDCOplus® Hemodynamic Monitor, LiDCO Ltd, London, UK]. A special software was installed to accommodate the LiDCOplus to measurements in large animals (LiDCOplus ver V4 Vet Configuration). Blood haemoglobin and plasma sodium concentration were entered into the LiDCOplus monitor. A bolus of 2.25 millimol (mmol) of lithium chloride (LiCl) was delivered manually through the drug catheter into the jugular vein. The LiCl was injected five seconds after initiating the measurement, to allow the 12 s of stable baseline required for accurate CO calculation (Hallowell et al. 2005). For the detection of the LiCl by the LiDCO sensor, arterial blood was withdrawn from the transverse facial artery by the LiDCO Flow Regulator.

### 4.3.2.6 Electrical stimulation

For determination of adequate anaesthetic depth, a constant current (CC) electrical stimulation was used (LEVIONNOIS et al. 2009). To deliver CC stimulation, two surface electrodes (Neuroline® 70005-J/12, Ambu GmbH, Bad Nauheim, Germany) were applied with an inter-electrode distance of 1 centimetre (cm) to the shaved and degreased skin over the lateral palmar digital nerve between the coronary band and the fetlock joint of the right forelimb. The electrodes were secured with an esmarch bandage. Stimuli consisted of a 25 ms train of five 1 ms CC 40 mA square-wave pulses. The trains of five were delivered at a frequency of 5 Hz. Directly before each stimulation, resistance between electrodes was measured (digital multimeter, VC260, Voltcraft®, Conrad Electronic SE, Hirschau, Germany). If the resistance was above 3 kΩ, the electrodes were repositioned to ensure discharge of a current of 40 mA.

### 4.3.2.7 Experimental protocol

After induction of anaesthesia, an endotracheal tube was placed and horses were hoisted on a padded surgery table in left lateral recumbency. Five min after induction,
TIVA was started (anaesthesia time zero). Drugs were administered with three separate syringe pumps (perfusor®compact, B. Braun Melsungen AG, Melsungen, Germany) to maintain anaesthesia. The animals were breathing spontaneously an air-oxygen mixture with an inspired oxygen fraction (FiO₂) of 0.5 - 0.6 from a large animal circle breathing system (Vet.-Tech. Modell JAVC-2000 J.D. Medical Distributing Company, Phoenix, USA). Ringer’s lactate (Ringer Ecobag®click, B.Braun Melsungen AG, Melsungen, Germany) was infused at a rate of 5 ml/kg bwt/h, IV. Electrical stimulation was performed first 30 min after induction and thereafter every 30 min. Ketamine infusion rate was increased by 0.5 mg/kg bwt/h in case of positive reaction to electrical stimulation or spontaneous movement. Thiopental (1 mg/kg bwt IV) [Trapanal®, Nycomed Deutschland GmbH, Konstanz, Germany] was given if the horse was trying to rise.

After 120 min of anaesthesia the infusion was discontinued, the total dose of anaesthetic drugs recorded and horses hoisted in a padded recovery box. Ten millilitres of phenylephrine [Phenylephrine 0,15%, Löwenapotheke, Hanover, Germany] were instilled into the nares to decongest the nasal cavities. During recovery, oxygen was insufflated at 15 L/min, initially through the endotracheal tube, after extubation through the ventral nasal meatus. All horses received flumazenil (0.01 mg/kg bwt IV) [Flumazenil HEXAL® 0,1 mg/ml Injektionslösung, HEXAL AG, Holzkirchen, Germany] 20 min after the end of TIVA. Duration of lateral and sternal recumbency and total time to standing position were recorded. Recovery quality was scored based on a previously published 100-point scoring system with 11 categories and a possible total score range of 11 to 100. A score of 11 would be an ideal recovery whereas a score of 100 would be the most uncoordinated, fractions recovery (CLARK-PRICE et al. 2008).

4.3.2.8 Cardiopulmonary measurements
Systolic, mean and diastolic arterial blood pressure, base-apex electrocardiogram (ECG), the inspired/ expired oxygen fraction (F₁O₂/ E₆O₂), the endtidal carbon dioxide fraction (E₆CO₂) and body temperature via a nasal probe were measured continuously (Cardiocap™/5, Datex-Ohmeda GmbH, Duisburg, Germany). Gas was
sampled from the proximal end of the endotracheal tube. Arterial blood samples for blood-gas analysis and acid-base status were withdrawn from the transverse facial artery into two ml heparinized plastic syringes and analysed immediately (ABL800 Flex, Radiometer GmbH, Willich, Germany). Qualitative parameters for anaesthetic depth (palpebral reflex, position of the eye, presence of nystagmus, muscle tone, swallowing reflex and spontaneous movements) were assessed by one anaesthetist.

Awake baseline measurements were performed after instrumentation; sedation values were determined five minutes after bolus administration of the tested \( \alpha_2 \)-adrenoceptor agonist and included HR, RR, arterial pressure readings and cardiac output determination. Values for RR, HR, \( \text{FiO}_2 \), \( \text{ET} \text{O}_2 \), \( \text{ET} \text{CO}_2 \), systolic, mean and diastolic arterial blood pressure, body temperature and qualitative parameters for anaesthetic depth were measured every 10 minutes from zero to 120 min. Arterial blood-gas and pH analysis and cardiac output measurement by lithium dilution technique were performed at 30, 60, 90 and 120 minutes.

Stroke volume (SV), stroke volume index (SVI), CI, arterial oxygen content (\( \text{CaO}_2 \)) and oxygen delivery (\( \text{DaO}_2 \)) were derived from standard formulas (KALCHOFNER et al. 2009):

\[
SV(\text{ml/beat}) = \frac{\text{CO} \times 1000}{\text{HR}},
\]

\[
SVI(\text{ml/beat/kg bwt}) = \frac{SV}{BW},
\]

\[
CI(\text{ml/kg bwt/min}) = \frac{\text{CO}}{BW}.
\]

\[
\text{CaO}_2(\text{ml/dL}) = (\text{Hb} \times 1.36 \times \text{SaO}_2) + (0.0031 \times \text{PaO}_2),
\]

haemoglobin (Hb) was measured in gram/decilitre (g/dl) and arterial oxygen saturation as a ratio

\[
\text{DaO}_2(\text{ml/kg bwt/min}) = CI \left( \frac{\text{CaO}_2}{100} \right).
\]

Alveolar dead space fraction \( (V_D) \) and oxygenation index \( (\text{OI}) \) were calculated as

\[
V_D = \frac{(P_a \text{CO}_2 - E_r \text{CO}_2)}{P_a \text{CO}_2}
\] and
\[OI = P_iO_2 / F_iO_2.\]

Systemic vascular resistance was estimated as follows (EDNER et al. 2005):

\[SVR (mmHg/L/min) = MAP (mmHg) / CO (L/min).\]

4.4 Statistical analysis

Goodness of fit for normal distribution of model residuals of data was performed by visual assessment of normal probability plots and the Shapiro-Wilk test. Characteristics of recovery from anaesthesia were neither normally nor lognormally distributed. For these data nonparametric methods were used. Normally distributed data are presented as mean ± SD. A two-way analysis with repeated measurements with post hoc Dunnett’s t-test was used to analyse changes in haemodynamic and respiratory data, NGD, skin temperature and thermal thresholds. Wilcoxon signed rank test for not normally distributed matched pairs was used to compare characteristics of recovery from anaesthesia between groups.

All analyses were carried out with the statistical software SAS, version 9.2 (SAS Institute, Cary, NC). For the analysis of the linear model, the mixed procedure was used. Values of \( p < 0.05 \) were considered significant.

4.5 Results

4.5.1 Pretrials

All horses developed signs of mild to deep sedation including moderate ataxia. After bolus administration no (X), two (D1) and one (D2) horse developed first-degree atrioventricular blocks (AV-blocks), and five (X), three (D1) and three (D2) horses developed second-degree AV-blocks. During infusion two (X), one (D1) and three (D2) horses showed first-degree AV-blocks and one (X), two (D1) and three (D2) horses had second degree AV-blocks. Heart rate was significantly decreased from baseline values over the majority of the 120 min infusion period with no significant differences among the groups (Table 1).

Respiratory rate decreased significantly from baseline over the whole 120 min treatment period in group D2 only (Table 1).
The NGD was significantly reduced from time 0 until 135 min, from time 5 until 120 min and from time 5 until 135 min in group X, group D1 and group D2, respectively (Figure 1).

Mean skin temperature remained between 33°C and 35°C in all groups (Table 2). Thermal thresholds increased significantly above baseline from time 15 and remained raised until 120 min in group X and D1 and until 150 min in group D2 (Figure 2). There was no significant difference among groups. The 95% confidence interval (CI) of TTs increased above the upper 95% CI of baseline measurements after 15 min in group X and D2.

4.5.2 Total intravenous anaesthesia

4.5.2.1 Anaesthesia

Induction of anaesthesia was excellent in all horses and endotracheal intubation was possible without problems. Mean required cumulative ketamine dose after 120 minutes of anaesthesia of group XKM, DKM1 and DKM2 were 3.7 ± 0.7 mg/kg bwt/h, 3.6 ± 0.5 mg/kg bwt/h and 3.7 ± 0.4 mg/kg bwt/h, respectively, and were not statistically different (Table 3).

4.5.2.2 Recovery

Recovery time in group DKM1 was significantly shorter than in group DKM2 (p = 0.049) while there was just a slight difference between XKM and DKM2 (p = 0.057). Recovery scores differed not significantly between groups (Table 3).

4.5.2.3 Haemodynamic variables

Heart rate decreased significantly after sedation and during anaesthesia in all three groups. Mean HR was continuously higher in group XKM (Table 4). There was an immediate increase in MAP after sedation, which did not reach significance. There was no significant difference from baseline during the anaesthetic period in all three groups (Figure 3). Cardiac index decreased significantly after sedation in all groups, and during the first hour of anaesthesia in group DKM2. With XKM CI was not different from baseline at all time points, but had a trend to increase continuously.
reaching higher values than baseline \( p = 0.068 \). During the whole anaesthetic period CI was significantly higher in group XKM compared to group DKM2 and DKM1 at time 60 and 90 (Figure 4). Stroke volume increased significantly at the end of anaesthesia in group XKM and DKM1. There was a significant difference between group XKM and DKM2 at time 60 and 120 (Table 5). Systemic vascular resistance increased significantly immediately after sedation in all groups, but remained raised at time 30 in group DKM2 resulting in a group difference between DKM1 and DKM2 at this time point. Over the whole anaesthetic period SVR was significantly lower in group XKM compared to group DKM2 (Figure 5). Oxygen delivery decreased significantly after sedation in all groups. Whereas \( \text{DaO}_2 \) returned to baseline values in group XKM, in groups DKM1 and DKM2 \( \text{DaO}_2 \) remained decreased during anaesthesia. In group XKM \( \text{DaO}_2 \) was significantly higher during the whole anaesthetic period than in group DKM2 (Table 5).

4.5.2.4 Temperature

The body temperature decreased throughout anaesthesia compared to baseline in all groups. The anaesthesia protocol had no effect on temperature. Mean values varied from 35.9 to 37.6 °C (Table 4).

4.5.2.5 Respiration

There was no significant difference in RR between anaesthetic protocols but RR varied largely between individuals, ranging from 3 to 25 breaths/min (Table 4). Arterial CO\(_2\) tension, arterial O\(_2\) tension and pH were similar in all groups (Table 5). Arterial CO\(_2\) tension increased significantly from baseline to anaesthesia in all groups, whereas arterial O\(_2\) tension and pH did not differ from baseline values. Oxygenation index and \( V_D \) did not change over time and were not different among groups (Table 6).

4.6 Discussion

Overall, the results of the current study demonstrated that an infusion of xylazine or dexmedetomidine at two different doses in combination with midazolam and
ketamine provided a cardiovascularly stable anaesthesia over two hours with similar recovery quality.

The first part of the study was designed to determine an equipotent dose of dexmedetomidine and xylazine suitable for constant rate infusion to be used as part of TIVA in horses. The extent and duration of head drop had been the main criterion to evaluate sedation in horses, which is an accepted method to assess $\alpha_2$-adrenoceptor agonist induced sedation (KAMERLING et al. 1988; BRYANT et al. 1991; BETTSCHART-WOLFENSBERGER et al. 1999b). The bolus of 0.5 mg/kg bwt followed by a CRI of 1 mg/kg bwt/h xylazine IV was set as the reference for depth of sedation. The common preanaesthetic xylazine dose before induction of anaesthesia with ketamine is 1.0 or 1.1 mg/kg bwt IV (GREENE et al. 1986; YOUNG et al. 1993; BETTSCHART-WOLFENSBERGER et al. 1996; MUIR et al. 2000; OKU et al. 2005; REZENDE et al. 2010). But clinical experience with our population of horses showed, that they became very atactic and one individual collapsed with this bolus dose so we decided to reduce the bolus of xylazine used for sedation to 0.5 mg/kg bwt IV. This xylazine bolus reduced NGD by 73% five minutes after the end of the injection. The bolus of 3.5 $\mu$g/kg bwt of dexmedetomidine resulted in a reduction of 43% and 64% in group DKM1 and DKM2, which is even less than the low dose of xylazine used in this study. The difference between head drop after the same dexmedetomidine bolus in group DKM1 and DKM2 might be related to differences in initial sympathetic tone of the horses. Since the trial with the higher dexmedetomidine dose was always the last trial the horses might have been familiarized with the trial set up resulting in less excitement allowing better sedation. In previous studies in ponies a dose of 3.5 $\mu$g/kg bwt dexmedetomidine was considered to be equisedative to medetomidine 7 $\mu$g/kg bwt or xylazine 1 mg/kg bwt (BETTSCHART-WOLFENSBERGER et al. 2005). In horses, 7.7 $\mu$g/kg bwt medetomidine and 1 mg/kg bwt xylazine were equisedative (YAMASHITA et al. 2000). If the horse population in the current study was overly sensitive to xylazine effects remains unknown.

To obtain a level of sedation equal to xylazine judged by NGD a dexmedetomidine CRI of 7 $\mu$g/kg bwt/h was necessary. This dose resulted in reduction of NGD by 70%
which was more than in ponies after a medetomidine bolus of 5 µg/kg followed by a CRI of 3.5 µg/kg/h inducing a constant head drop of 50% for two hours (BETTSCHART-WOLFENSBERGER et al. 1999b; BETTSCHART-WOLFENSBERGER et al. 2005). Considering the potency ratio of 1:2 between medetomidine and dexmedetomidine on receptor binding studies (VIRTANEN 1989), the high dose of dexmedetomidine for CRI used in the current study was four times higher than the dose used in the ponies.

The α₂-adrenoceptor agonist drugs produce excellent analgesia in horses (KAMERLING et al. 1988; ENGLAND and CLARKE 1996). However, the analgesic potencies of various α₂-adrenoceptor agonists differ depending upon the species and analgesic test employed as well as the ratio between analgesia to sedation (ENGLAND and CLARKE 1996; KÄSTNER 2006). Due to the complexity of nociceptive pathways and nociceptive receptor diversity, different noxious stimuli (i.e. thermal, mechanical, chemical, electrical) are required to fully evaluate the antinociceptive effect of drugs. Noxious thermal stimulation is considered to be an objective and repeatable method for assessing cutaneous analgesia in horses (KAMERLING et al. 1985; KAMERLING et al. 1988; ROBERTSON et al. 2005). While noxious electrical stimulation of the skin preferentially activates small myelinated Aδ fibers which are responsible for the initiation of pain (“first pain”), noxious thermal stimuli activate additionally slowly conducting C-fibers, which have been associated with persistent clinical or chronic pain (GAYNOR and MUIR 2009).

Results from the present study demonstrated analgesia following xylazine and dexmedetomidine in two different dosing regimes. Although there was no significant difference between groups, mean values for thermal threshold after low dose dexmedetomidine were consistently lower compared with the two other treatments. On the other hand, reaction to the thermal stimulation at the area over the right nostril implicates movement of the head or at least the ears which might be influenced by the muscle relaxant effect of α₂-adrenoceptor agonists. Further a high density of α₂-adrenoceptor agonists have been demonstrated in lamina II of the dorsal horn of the spinal cord (BOUCHENAF and LIVINGSTON 1987), which is an area widely implicated in the transmission of painful stimuli. Noxious stimulation at the area over
the right nostril is transmitted via the sensory infraorbital nerve as part of the trigeminal nerve and does not involve the dorsal horn of the spinal cord. Thus, produced analgesia must basically result from activation of $\alpha_2$-adrenoceptors located in the locus coeruleus and the periaqueductal gray mater (GUO et al. 1996; PENG et al. 1996; BUDAI et al. 1998). However, results of thermal stimulation with the same test system as used in the current study at withers (=spinal) and nostrils are not different (POLLER 2011, study in preparation).

Cardiopulmonary changes produced by different $\alpha_2$-adrenoceptor agonists in horses are similar (ENGLAND and CLARKE 1996). The haemodynamic effects include the typical biphasic blood pressure response with decreased heart rate and cardiac index as well as an increase in systemic vascular resistance (ENGLAND and CLARKE 1996). In the current sedation trial, the heart rate decreased significantly after bolus injection and remained low with CRI of xylazine as well as both dexmedetomidine doses. In contrast, BETTSCHART-WOLFENSBERGER et al. 2005 reported no significant reduction in heart rate with a dose of 3.5 $\mu$g/kg bwt dexmedetomidine IV in ponies. Studies in man suggest that increasing concentrations of dexmedetomidine during CRI resulted in a decrease in HR, a progressive decrease in CO, and a biphasic (low, then high) dose-response relation for arterial blood pressure and vascular resistance because of peripheral vaso- and venoconstriction, so that cardiovascular effects might limit the usefulness of high concentrations of dexmedetomidine (EBERT et al. 2000).

With respect to the high dose of dexmedetomidine required to obtain the same degree of head drop (sedation and muscle relaxation) as with xylazine, we decided to test a CRI of 1 mg/kg bwt/h of xylazine and both dexmedetomidine doses with 5 $\mu$g/kg bwt/h and 7 $\mu$g/kg bwt/h in combination with a starting dose of 3 mg/kg bwt/h of ketamine and 0.1 mg/kg bwt/h of midazolam for 120 minutes TIVA.

Our data suggest that cardiovascular function is maintained within acceptable limits for horses with all three protocols. The determination of CO is used as a global indicator of central perfusion and to assess the effect of drugs on circulation. Studies on various combinations of $\alpha_2$-adrenoceptor agonists (xylazine, detomidine,
romifidine, medetomidine), dissociative anaesthetics (ketamine, tiletamine) and central muscle relaxants (guaifenesin, diazepam, clima zolam, midazolam, zolazepam) for TIVA in horses demonstrated less cardiovascular depression compared with inhalation anaesthesia (GREENE et al. 1986; BETTSCHART-WOLFENSBERGER et al. 1996; KERR et al. 1996; MCMURPHY et al. 2002; MAMA et al. 2005). In detail, a guaifenesin (137.5 mg/kg bwt/h), ketamine (2.75 mg/kg bwt/h) and xylazine (1.375 mg/kg bwt/h) TIVA over 120 min decreased CI to 65% of baseline value at five minutes after induction with guaifenesin, ketamine and xylazine and returned immediately to baseline value 15 min thereafter (GREENE et al. 1986). With detomidine (32, 24 and 16 µg/kg bwt/h), ketamine (3.2, 2.4 and 1.6 mg/kg bwt/h) and guaifenesin (80, 60 and 40 mg/kg bwt/h) CI decreased significantly after induction and during 120 min of anaesthesia to approximately 75% (LUNA et al. 1996). A midazolam (0.08 mg/kg bwt/h), ketamine (4 mg/kg bwt/h), medetomidine (10 µg/kg bwt/h) TIVA over 60 min decreased CI to 70-80% of awake baseline values (YAMASHITA et al. 2007). A propofol (0.14 mg/kg bwt/h), ketamine (1 mg/kg bwt/h), medetomidine (1.25 µg/kg bwt/h) TIVA resulted in a reduction to 65-70% of baseline (UMAR et al. 2007), whereas a prolonged anaesthesia over four hours via propofol (4.2 – 6.6 mg/kg bwt/h) and medetomidine (3.5 µg/kg bwt/h) reduced CI to 50-70% (BETTSCHART-WOLFENSBERGER et al. 2001c). With a clima zolam (0.4 mg/kg bwt/h)-ketamine (6 mg/kg bwt/h) infusion over 120 min CI decreased to 65% of baseline immediately after induction and remained constant over the maintenance period (BETTSCHART-WOLFENSBERGER et al. 1996). In our study CI differed between groups. In the XKM-TIVA CI increased over the duration of anaesthesia reaching higher values than at baseline (130% of baseline), whereas with the high dose of dexmedetomidine CI was decreased to 78% of baseline during anaesthesia, indicating that the type and dose of α₂-adrenoceptor agonists used with TIVA protocols significantly influence cardiac performance and perfusion. It is well known that α₂-adrenoceptor agonists cause an initial hypertension followed by a prolonged hypotension together with a reduced CI and an increased SVR after bolus administration (KERR et al. 1972; WAGNER et al. 1991; BETTSCHART-WOLFENSBERGER et al. 1999a; YAMASHITA et al. 2000; KÅSTNER et al. 2001;
MURRELL and HELLEBREKERS 2005; KÄSTNER 2006). To our knowledge, there are no data available that directly compare the influence of different \(\alpha_2\)-adrenoceptor agonists on CI during TIVA. The increase in CI in the course of XKM-TIVA is surprising. This increase was attributable to a rise in SV. Stroke volume is determined by preload, cardiac contractility, and afterload. Xylazine caused transient reductions in cardiac contractility, dependent on dose and route of administration (WAGNER et al. 1991). It produced vasoconstriction in the isolated canine pulmonary vein providing evidence that \(\alpha_2\)-adrenoceptor agonists might increase preload (HANIUDA et al. 1989). After IV administration of xylazine or dexmedetomidine in horses preload increased (WAGNER et al. 1991). Stimulation of postsynaptic \(\alpha_2\)-adrenergic receptors in the vascular smooth muscle is responsible for an increase in vascular tone after intravenous administration of \(\alpha_2\)-adrenoceptor agonists (BRYANT et al. 1996; LINK et al. 1996; BRYANT et al. 1998). As SVR decreased over time in the XKM group a reduction of afterload was indicated. However, hypotensive effects of \(\alpha_2\)-adrenoceptor agonists are centrally mediated. It is not known, whether central sympatholytic effects or peripheral vascular action of xylazine predominate over time when given as a constant rate infusion. Dexmedetomidine is not a pure \(\alpha_2\)-adrenoceptor agonist but also acts at imidazoline receptors (xylazine does not) that mediate central hypotension and anti-arrhythmogenic action (HIEBLE and RUFFOLO 1995; FABER et al. 1998) and may be responsible for the lower MAP with DKM1. Further, the selectivity of dexmedetomidine is greater for the \(\alpha_2\)-adrenoceptor than the \(\alpha_1\)-adrenoceptor compared to xylazine (WAGNER et al. 1991; AANTAA et al. 1993) and it is known, that mixed \(\alpha_1\)/\(\alpha_2\)-adrenoceptor agonists produce more potent vasoconstriction than \(\alpha_2\)-adrenoceptor agonists (SKRBIC and CHIBA 1993; IIDA et al. 1999). A cause of the difference in MAP between DKM1 and DKM2 may be the different cardiovascular effects of dexmedetomidine at low and high plasma concentrations. In humans, low plasma concentrations (0.7 to 1.2 ng/ml) decreased arterial blood pressure, perhaps because concentrations were below threshold to produce significant peripheral vasoconstriction, or the sympatholytic effects of dexmedetomidine offset any direct effect on the peripheral vasculature. Higher
plasma concentrations (>1.9 ng/ml) resulted in a progressive increase in BP and may be an explanation of the higher MAP and SVR with DKM2 (EBERT et al. 2000).

Respiratory effects were similar between the three TIVA protocols. We decided to give supplemental oxygen with an inspired oxygen fraction of 50% because during TIVA with the horse’s breathing room air hypoxaemia is well documented (KERR et al. 1996; YAMASHITA et al. 2007). In this study, no horse became hypoxaemic, but in 3 horses inspired oxygen fraction had to be elevated to 75% to maintain arterial O2 tension above 70 mmHg.

At the beginning, the level of anaesthesia did not represent a surgical plane of anaesthesia because almost all horses responded with purposeful movements to nociceptive electrical stimulation. Therefore, the starting dose of 3 mg/kg bwt/h of ketamine would only allow diagnostic procedures. A ketamine infusion rate between 4 and 4.5 mg/kg bwt/h was sufficient to suppress purposeful movement in response to electrical stimulation in all three groups. In the preliminary trial, xylazine, 1 mg/kg bwt/h, and dexmedetomidine, 7 µg/kg bwt/h, had very similar effects on thermal thresholds, whereas the lower dose of dexmedetomidine was less effective in increasing thermal thresholds. This was not reflected in higher ketamine requirements during TIVA. As mentioned above, noxious electrical and thermal stimulation of the skin activates different fibers which are responsible for the initiation of first pain or chronic pain (GAYNOR and MUIR 2009). In addition, the lack of responses to noxious electrical stimulation during anaesthesia not only assesses nociception but also hypnosis and immobility.

The quality of recovery ranged from excellent to good in our study with no differences between groups. Previously described protocols for TIVA using midazolam or other benzodiazepine agonists as muscle relaxant resulted in various degrees of ataxia after standing. (MUIR et al. 2000; GANGL et al. 2001; YAMASHITA et al. 2007). The duration of action of all benzodiazepines is strongly dependent on the duration of their administration (OLKKOLA and AHONEN 2008). Recovery from anaesthesia after bolus administration of benzodiazepines is enhanced by redistribution, but is slower after long-lasting infusion of benzodiazepines for maintenance (OLKKOLA a.
AHONEN 2008). This disadvantage for prolonged use in TIVA might be counteracted by the use of specifically acting benzodiazepine antagonists. The elimination half-lives and potencies of both, agonists and antagonists, are important to avoid ataxic recoveries and resedation. The benzodiazepine antagonist sarmazenil in a dose of 0.04 mg/kg bwt IV has been used to overcome ataxia in horses anaesthetized with ketamine (6 mg/kg bwt/h IV) and climazolam (0.4 mg/kg bwt/h IV) over 120 minutes, but two out of six horses were atactic during recovery (BETTSCHART-WOLFENSBERGER et al. 1996). This behaviour during recovery may be related to high plasma ketamine concentrations or to inadequate reversal of climazolam. WETTSTEIN et al. (2006) reported a quick anduneventful recovery in ponies after anaesthesia maintained with detomidine (0.024 mg/kg bwt/h IV), climazolam (0.036 mg/kg bwt/h IV) and ketamine (2.4 mg/kg bwt/h IV) over 120 min followed by the benzodiazepine antagonist sarmazenil (0.04 mg/kg bwt IV). Climazolam and sarmazenil doses were almost the same as in the former study, but ketamine was less than half the dose, which might influence recovery quality. Resedation was not observed although elimination half-time of climazolam (5.1 h) is more than threefold longer than of sarmazenil (1.6 h) in horses (LUDWIG et al. 1984). To our knowledge no pharmacological information concerning elimination of midazolam or flumazenil exists for horses. In humans, the elimination half-life of midazolam ranges from 1.7 to 3.5 h (ALLONEN et al. 1981; HEIZMANN et al. 1983; GREENBLATT et al. 1984; MISAKA et al. 2010) and of flumazenil from 40 to 80 min (OLKKOLA and AHONEN 2008). The onset of effect immediately follows the diffusion of the substance into the CNS and can be observed within the first minutes following flumazenil administration (AMREIN and HETZEL 1990).

Dose rates of 0.07 mg/kg bwt/h and 0.08 mg/kg bwt/h of midazolam for central muscular relaxation during TIVA in horses without its reversal are reported (BOUTS et al. 2001; YAMASHITA et al. 2007). Based on these reports and clinical observations, in the current study it was decided to administer 0.1 mg/kg bwt/h of midazolam followed by its reversal with 0.01 mg/kg bwt of flumazenil. To the authors knowledge there are no reports on the use of flumazenil after CRI of midazolam in horses. A range of 0.01 to 0.05 mg/kg bwt of flumazenil is recommended for
benzodiazepine reversal in horses (MUIR 2009a). In dogs, a dose of 0.04 mg/kg bwt IV of flumazenil was successfully used to antagonize midazolam-induced changes in the EEG (KEEGAN et al. 1993). In humans, flumazenil improves the level of consciousness in patients with benzodiazepine overdose. However, resedation may occur within one to two h after administration and repeated doses or a continuous infusion may be necessary (HOFFMAN 1993). During our study, we observed no signs of resedation or ataxia during or after recovery.

Overall, recovery quality can be influenced by plasma concentration of anaesthetic drugs, in turn its pharmacokinetic properties as well cardiovascular function that may affect its clearance (DUTTA et al. 2000). Despite the necessity of complete reversal of midazolam, sedation must be sufficient to allow plasma concentrations of ketamine to decline, but should not prolong recovery time. In all groups, flumazenil was given 20 min after the end of TIVA, but recovery time was significantly longer with DKM2 compared with DKM1. The duration of the sedative effect of α2-adrenoceptor agonists depends upon dose and duration of infusion. Although recovery time was longer with DKM2, there was no difference in recovery quality, so that the higher dose of dexmedetomidine was not beneficial.

The study design has some limitation. During the pretrials, horses were assigned to group X or D1 in randomised order, but horses in group D2 were all sedated two times before and were therefore familiar with the procedure. As mentioned before, an effect of acclimatisation cannot be excluded. There were no clinical signs of ketamine or norketamine cumulation after two hours of TIVA. However, the ketamine dose was increased from 3 mg/kg/h every 30 min if required and results indicate that 4 mg/kg/h are necessary to achieve immobility after electrical stimulation. Therefore, cumulative effects of a higher ketamine dose from the beginning of TIVA and its influence on recovery quality cannot be ruled out. In the current study the three different drugs were infused separately to allow independent change of infusion rates. For practical clinical use, the chemical stability and compatibility of a combination of the drugs in fluid bag should be determined.
In conclusion, cardiopulmonary function was well maintained with excellent quality of recovery with all three protocols. The higher dose of dexmedetomidine resulted in more cardiovascular depression and a longer recovery period, but no reduction of the ketamine requirements. Therefore the higher dose has no advantage above the lower dose. Xylazine is a licensed drug for horses, whereas dexmedetomidine implies off licence use. A ketamine dose of at least 4 mg/kg bwt/h IV was required to obtain no response to stimulation, whereas 3 mg/kg bwt/h IV ketamine might be enough for non painful diagnostic procedures.
## 4.7 Tables and Figures

Table 1: Heart rate and respiratory rate (mean ± SD) in 5 horses after xylazine (0.5 mg/kg bwt and 1 mg/kg/h) [X], dexmedetomidine (3.5 µg/kg bwt and 5 µg/kg/h) [D1] or dexmedetomidine (3.5 µg/kg bwt and 7 µg/kg/h) [D2] from 0 to 120 min.

<table>
<thead>
<tr>
<th>time</th>
<th>HR (min)</th>
<th>RR (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>D1</td>
</tr>
<tr>
<td>baseline</td>
<td>39 ± 7</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>0</td>
<td>24 ± 0a</td>
<td>28 ± 6a</td>
</tr>
<tr>
<td>5</td>
<td>29 ± 4a</td>
<td>29 ± 4a</td>
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<tr>
<td>10</td>
<td>31 ± 5a</td>
<td>32 ± 4</td>
</tr>
<tr>
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<td>33 ± 3</td>
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<td>32 ± 3</td>
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<td>32 ± 4</td>
</tr>
<tr>
<td>70</td>
<td>31 ± 3a</td>
<td>31 ± 2a</td>
</tr>
<tr>
<td>75</td>
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</tr>
<tr>
<td>80</td>
<td>31 ± 3a</td>
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<td>31 ± 2a</td>
</tr>
<tr>
<td>100</td>
<td>30 ± 3a</td>
<td>30 ± 2a</td>
</tr>
<tr>
<td>105</td>
<td>31 ± 3a</td>
<td>32 ± 3</td>
</tr>
<tr>
<td>110</td>
<td>30 ± 3a</td>
<td>31 ± 4a</td>
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<tr>
<td>115</td>
<td>31 ± 3a</td>
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<tr>
<td>120</td>
<td>30 ± 3a</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>135</td>
<td>35 ± 4</td>
<td>33 ± 1</td>
</tr>
<tr>
<td>150</td>
<td>38 ± 6</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>180</td>
<td>36 ± 4</td>
<td>36 ± 3</td>
</tr>
</tbody>
</table>

a Significantly (p < 0.05) different from baseline. b Significant (p < 0.05) difference between group X and D2. c Significant (p < 0.05) difference between group D1 and D2.
Table 2: Skin temperature (mean ± SD) in 5 horses after xylazine (0.5 mg/kg bwt and 1 mg/kg/h) [X], dexmedetomidine (3.5 µg/kg bwt and 5 µg/kg/h [D1] or dexmedetomidine (3.5 µg/kg bwt and 7 µg/kg/h [D2] from 0 to 120 min.

<table>
<thead>
<tr>
<th>Skin temperature</th>
<th>X</th>
<th>D1</th>
<th>D2</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>34.1 ± 0.6</td>
<td>34.7 ± 0.8</td>
<td>33.2</td>
</tr>
<tr>
<td>15</td>
<td>34.6 ± 0.5</td>
<td>34.9 ± 0.6</td>
<td>34.9a</td>
</tr>
<tr>
<td>30</td>
<td>34.4 ± 0.7</td>
<td>34.9 ± 0.8</td>
<td>33.7</td>
</tr>
<tr>
<td>60</td>
<td>33.2 ± 0.6a</td>
<td>35.1 ± 0.8</td>
<td>32.7</td>
</tr>
<tr>
<td>90</td>
<td>33.5 ± 1.0</td>
<td>34.7 ± 0.9</td>
<td>32.8</td>
</tr>
<tr>
<td>120</td>
<td>33.4 ± 0.7</td>
<td>34.6 ± 0.8</td>
<td>32.5</td>
</tr>
<tr>
<td>150</td>
<td>33.5 ± 0.7</td>
<td>34.3 ± 0.7</td>
<td>33.0</td>
</tr>
<tr>
<td>180</td>
<td>33.3 ± 0.5</td>
<td>34.5 ± 0.8</td>
<td>33.0</td>
</tr>
</tbody>
</table>

Reading at baseline = mean of four pre-treatment measurements. a significantly (p < 0.05) different than baseline value.

Table 3: Mean recovery time, recovery quality and required ketamine dose in 8 horses after anaesthesia with a CRI of xylazine (1 mg/kg bwt/h), [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1], or dexmedetomidine (7 µg/kg bwt/h) [DKM2] in combination with a variable ketamine and midazolam (0.1 mg/kg bwt/h IV).

<table>
<thead>
<tr>
<th></th>
<th>XKM</th>
<th>DKM1</th>
<th>DKM2</th>
</tr>
</thead>
<tbody>
<tr>
<td>recovery time</td>
<td>37.1 ± 16.1</td>
<td>31.5 ± 8.9a</td>
<td>45.5 ± 20.9a</td>
</tr>
<tr>
<td>(min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recovery quality</td>
<td>32.5 ± 14.5</td>
<td>24.4 ± 13.6</td>
<td>20.9 ± 10.9</td>
</tr>
<tr>
<td>(score points)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ketamine dose</td>
<td>3.7 ± 0.7</td>
<td>3.6 ± 0.5</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>(mg/kg bwt/h)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD. a Significant difference (p < 0.05) between group DKM1 and DKM2.
Table 4: Mean heart rate, respiratory rate and body temperature (Temp) before treatment (baseline), immediately after sedation (sedation) and anaesthetized in horses with a CRI of xylazine (1 mg/kg bwt/h), [XKM] or dexmedetomidine (5 μg/kg bwt/h) [DKM1], or dexmedetomidine (7 μg/kg bwt/h) [DKM2] in combination with a variable rate of ketamine and midazolam (0.1 mg/kg bwt/h IV).

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>HR (min)</th>
<th>RR (min)</th>
<th>Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>baseline</td>
<td>XKM</td>
<td>38 ± 4</td>
<td>14 ± 2</td>
<td>37.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>DKM1</td>
<td>38 ± 5</td>
<td>14 ± 2</td>
<td>37.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>38 ± 3</td>
<td>14 ± 2</td>
<td>37.5 ± 0.2</td>
</tr>
</tbody>
</table>

|       |         |          |          |           |
| sedation | XKM  | 30 ± 2<sup>a</sup> | 10 ± 2   | 37.8 ± 0.2 |
|         | DKM1 | 31 ± 6<sup>a</sup> | 11 ± 2   | 37.4 ± 0.2 |
|         | DKM2 | 30 ± 3<sup>a</sup> | 11 ± 1   | 37.5 ± 0.2 |

|       |         |          |          |           |
| 10     | XKM  | 32 ± 5<sup>a,x</sup> | 11 ± 5   | 36.1 ± 0.6 |
|         | DKM1 | 29 ± 3<sup>a</sup>   | 11 ± 6   | 36.6 ± 0.1 |
|         | DKM2 | 27 ± 2<sup>a,x</sup> | 16 ± 7   | 36.2 ± 0.6 |

|       |         |          |          |           |
| 20     | XKM  | 31 ± 4<sup>a</sup>   | 11 ± 5   | 36.1 ± 0.5 |
|         | DKM1 | 29 ± 3<sup>a</sup>   | 11 ± 6   | 36.8± 0.1  |
|         | DKM2 | 28 ± 3<sup>a</sup>   | 16 ± 6   | 36.3 ± 0.5 |

|       |         |          |          |           |
| 30     | XKM  | 31 ± 3<sup>a</sup>   | 12 ± 7   | 36.3 ± 0.5 |
|         | DKM1 | 29 ± 4<sup>a</sup>   | 12 ± 6   | 36.8 ± 0.2 |
|         | DKM2 | 29 ± 3<sup>a,b</sup> | 13 ± 5   | 36.3 ± 0.5 |

|       |         |          |          |           |
| 40     | XKM  | 32 ± 4<sup>a</sup>   | 11 ± 7   | 36.2 ± 0.6 |
|         | DKM1 | 30 ± 3<sup>a</sup>   | 13 ± 6   | 36.7 ± 0.2 |
|         | DKM2 | 29 ± 2<sup>a</sup>   | 16 ± 6   | 36.3 ± 0.5 |

|       |         |          |          |           |
| 50     | XKM  | 31 ± 3<sup>a</sup>   | 12 ± 5   | 36.3 ± 0.7 |
|         | DKM1 | 29 ± 4<sup>a</sup>   | 13 ± 5   | 36.7 ± 0.3 |
|         | DKM2 | 29 ± 3<sup>a</sup>   | 15 ± 6   | 36.2 ± 0.5 |

|       |         |          |          |           |
| 60     | XKM  | 31 ± 3<sup>a,x</sup> | 13 ± 6   | 36.1 ± 0.7 |
|         | DKM1 | 28 ± 4<sup>a</sup>   | 12 ± 4   | 36.7 ± 0.3 |
|         | DKM2 | 28 ± 2<sup>a,x</sup> | 13 ± 7   | 36.2 ± 0.6 |

|       |         |          |          |           |
| 70     | XKM  | 31 ± 4<sup>a,x</sup> | 13 ± 6   | 36.0 ± 0.7 |
|         | DKM1 | 28 ± 4<sup>a</sup>   | 12 ± 4   | 36.7 ± 0.3 |
|         | DKM2 | 28 ± 2<sup>a,x</sup> | 13 ± 5   | 36.2 ± 0.6 |

|       |         |          |          |           |
| 80     | XKM  | 30 ± 3<sup>a</sup>   | 12 ± 6   | 36.0 ± 0.7 |
|         | DKM1 | 27 ± 4<sup>a</sup>   | 11 ± 4   | 36.6 ± 0.3 |
|         | DKM2 | 29 ± 3<sup>a</sup>   | 14 ± 4   | 36.1 ± 0.6 |

|       |         |          |          |           |
| 90     | XKM  | 31 ± 3<sup>a,x</sup> | 11 ± 5   | 36.0 ± 0.7 |
|         | DKM1 | 28 ± 4<sup>a</sup>   | 13 ± 3   | 36.6 ± 0.4 |
|         | DKM2 | 28 ± 2<sup>a,x</sup> | 10 ± 4   | 36.1 ± 0.7 |

|       |         |          |          |           |
| 100    | XKM  | 31 ± 3<sup>a,x</sup> | 12 ± 7   | 35.9 ± 0.7 |
|         | DKM1 | 28 ± 4<sup>a</sup>   | 12 ± 4   | 36.6 ± 0.4 |
|         | DKM2 | 29 ± 2<sup>a,x</sup> | 13 ± 6   | 36.0 ± 0.7 |

|       |         |          |          |           |
| 110    | XKM  | 30 ± 4<sup>a</sup>   | 10 ± 5   | 36.7 ± 0.7 |
|         | DKM1 | 28 ± 4<sup>a</sup>   | 11 ± 3   | 36.5 ± 0.4 |
|         | DKM2 | 28 ± 2<sup>a</sup>   | 14 ± 9   | 36.0 ± 0.6 |

|       |         |          |          |           |
| 120    | XKM  | 31 ± 3<sup>a</sup>   | 9 ± 4    | 35.9 ± 0.7 |
|         | DKM1 | 28 ± 3<sup>a</sup>   | 13 ± 3   | 36.5 ± 0.4 |
|         | DKM2 | 28 ± 2<sup>a</sup>   | 12 ± 6   | 36.0 ± 0.6 |

Values are mean ± SD. <sup>a</sup> Significantly (p < 0.05) different than baseline value. <sup>b</sup> Significantly (p < 0.05) different than 10 minute value. <sup>c</sup> Significant (p < 0.05) difference between group XKM and DKM1. <sup>d</sup> Significant (p < 0.05) difference between group XKM and DKM2.
Table 5: Mean stroke volume and arterial blood gas analyses before treatment (baseline), immediately after sedation (sedation) and in anaesthetized horses with a CRI of xylazine (1 mg/kg bwt/h), [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1], or dexmedetomidine (7 µg/kg bwt/h) [DKM2] in combination with a variable rate of ketamine and midazolam (0.1 mg/kg bwt/h IV).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>group</th>
<th>SV (ml/beat)</th>
<th>pH</th>
<th>paO₂ (kPa)</th>
<th>paCO₂ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>XKM</td>
<td>1210 ± 560</td>
<td>7.4  ± 0.02</td>
<td>13.6 ± 0.4</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>DKM1</td>
<td>1172 ± 441</td>
<td>7.38 ± 0.02</td>
<td>13.3 ± 0.0</td>
<td>5.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>1185 ± 363</td>
<td>7.39 ± 0.02</td>
<td>13.3 ± 0.0</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>sedation</td>
<td>XKM</td>
<td>1065 ± 242</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DKM1</td>
<td>1127 ± 506</td>
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</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>1080 ± 296</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>XKM</td>
<td>1543 ± 631</td>
<td>7.38 ± 0.02</td>
<td>10.6 ± 1.9</td>
<td>7.2 ± 0.3 a</td>
</tr>
<tr>
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<td>DKM1</td>
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<td>18.5 ± 7.4</td>
<td>7.3 ± 0.3 a</td>
</tr>
<tr>
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<td>DKM2</td>
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<td>6.8 ± 0.4 a</td>
</tr>
<tr>
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<td>XKM</td>
<td>1807 ± 458 a</td>
<td>7.38 ± 0.02</td>
<td>16.9 ± 6.8</td>
<td>7.2 ± 0.3 a</td>
</tr>
<tr>
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<td>DKM1</td>
<td>1381 ± 380</td>
<td>7.38 ± 0.03</td>
<td>16.2 ± 6.3</td>
<td>7.3 ± 0.3 a</td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>1194 ± 174 a</td>
<td>7.38 ± 0.03</td>
<td>14.1 ± 5.9</td>
<td>6.9 ± 0.4 a</td>
</tr>
<tr>
<td>90</td>
<td>XKM</td>
<td>2024±1023 a</td>
<td>7.37 ± 0.03</td>
<td>15.6 ± 5.9</td>
<td>7.6 ± 0.7 a</td>
</tr>
<tr>
<td></td>
<td>DKM1</td>
<td>1424 ± 361</td>
<td>7.38 ± 0.03</td>
<td>17.6 ± 8.3</td>
<td>7.5 ± 0.4 a</td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>1429 ± 357</td>
<td>7.39 ± 0.02</td>
<td>13.7 ± 4.7</td>
<td>6.9 ± 0.4 a</td>
</tr>
<tr>
<td>120</td>
<td>XKM</td>
<td>2009 ± 769 a</td>
<td>7.38 ± 0.03</td>
<td>15.5 ± 4.8</td>
<td>7.7 ± 0.7 a</td>
</tr>
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<td>DKM1</td>
<td>1627 ± 425 a</td>
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<td>18.2 ± 8.7</td>
<td>7.4 ± 0.6 a</td>
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<td>DKM2</td>
<td>1284 ± 140 a</td>
<td>7.39 ± 0.01</td>
<td>14.9 ± 5.9</td>
<td>7.1 ± 0.4 a</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Time [minutes]; SV, stroke volume [ml/beat]; paO₂, arterial oxygen tension [kPa]; paCO₂, arterial carbon dioxide tension [kPa].

a Significantly (p < 0.05) different than baseline value.  * Significant (p < 0.05) difference between group XKM and DKM1.  # Significant (p < 0.05) difference between group XKM and DKM2. Significant (p < 0.05) difference between group DKM1 and DKM2.
Table 6: Oxygenation index, alveolar dead space fraction and oxygen delivery before treatment (baseline), immediately after sedation (sedation) and in anaesthetized horses with a CRI of xylazine (1 mg/kg bwt/h), [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1], or dexmedetomidine (7 µg/kg bwt/h) [DKM2] in combination with a variable rate ketamine and midazolam (0.1 mg/kg bwt/h IV).

<table>
<thead>
<tr>
<th>Time</th>
<th>group</th>
<th>OI (kPa)</th>
<th>V(_D) (1/1)</th>
<th>DaO(_2) (ml/kg bwt/minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XKM</td>
<td>64.4 ± 2.0</td>
<td>ND</td>
<td>15 ± 6</td>
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<tr>
<td></td>
<td>DKM1</td>
<td>63.9 ± 1.9</td>
<td>ND</td>
<td>16 ± 11</td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>64.1 ± 1.7</td>
<td>ND</td>
<td>14 ± 4</td>
</tr>
<tr>
<td>baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>XKM</td>
<td>29.3 ± 11.7</td>
<td>0.21 ± 0.10</td>
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<tr>
<td></td>
<td>DKM1</td>
<td>32.7 ± 12.1</td>
<td>0.15 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>25.3 ± 9.4</td>
<td>0.13 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>XKM</td>
<td>26.9 ± 10.9</td>
<td>0.20 ± 0.08</td>
<td>12 ± 4(^a)</td>
</tr>
<tr>
<td></td>
<td>DKM1</td>
<td>27.5 ± 11.4</td>
<td>0.18 ± 0.10</td>
<td>10 ± 5(^a)</td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>22.6 ± 8.6</td>
<td>0.14 ± 0.05</td>
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<td>90</td>
<td>XKM</td>
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<td>0.16 ± 0.10</td>
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<td></td>
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<td>10 ± 6(^a)</td>
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<td>120</td>
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<td>0.16 ± 0.10</td>
<td>14 ± 4(^a)</td>
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<tr>
<td></td>
<td>DKM1</td>
<td>27.9 ± 11.6</td>
<td>0.16 ± 0.06</td>
<td>10 ± 5(^a)</td>
</tr>
<tr>
<td></td>
<td>DKM2</td>
<td>22.6 ± 9.3</td>
<td>0.16 ± 0.06</td>
<td>8 ± 3(^a,#)</td>
</tr>
</tbody>
</table>

Values are mean ± SD. OI, oxygenation index [kPa]; V\(_D\), alveolar dead space fraction [1/1]; DaO\(_2\), oxygen delivery [ml/kg bwt/minute]. For V\(_D\) baseline calculation E\(_T\)CO\(_2\) was assumed as 5.3 kPa.
Figure 1: Mean (SD) nose-to-ground-distance (NGD) in 5 horses after xylazine (0.5 mg/kg bwt and 1 mg/kg/h) [X], dexmedetomidine (3.5 µg/kg bwt and 5 µg/kg/h) [D1] or dexmedetomidine (3.5 µg/kg bwt and 7 µg/kg/h) [D2] from 0 to 120 min.
Figure 2: Thermal threshold (mean, CI) temperature in 5 horses after xylazine (0.5 mg/kg bwt and 1 mg/kg/h) [X], dexmedetomidine (3.5 µg/kg bwt and 5 µg/kg/h) [D1] or dexmedetomidine (3.5 µg/kg bwt and 7 µg/kg/h) [D2] from 0 to 120. Reading at time 0 = mean of four pre-treatment measurements. 95% CI of pre treatment thresholds.
Figure 3: Mean ± SD mean arterial blood pressure (MAP) before treatment (-15min), immediately after sedation (-6 min) and in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1], or dexmedetomidine (7 µg/kg bwt/h) [DKM2] in combination with a variable rate ketamine and midazolam (0.1 mg/kg bwt/h IV).
Figure 4: Mean ± SD cardiac index (CI) before treatment (-15min), immediately after sedation (-6 min) and in anaesthetized horses with a CRI of xylazine (1 mg/kg bwt/h), [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1], or dexmedetomidine (7 µg/kg bwt/h) [DKM2] in combination with a variable rate ketamine and midazolam (0.1 mg/kg bwt/h IV).
Figure 5: Mean ± SD systemic vascular resistance (SVR) before treatment (-15min), immediately after sedation (-6 min) and in anaesthetized horses with a CRI of xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1], or dexmedetomidine (7 µg/kg bwt/h) [DKM2] in combination with a variable rate ketamine and midazolam (0.1 mg/kg bwt/h IV).

REFERENCES see page 115 and the following
5 Manuskript II

Pharmacokinetics of total intravenous anaesthesia using xylazine or dexmedetomidine, midazolam and ketamine in horses

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5.1 Abstract

Objectives: To compare elimination pharmacokinetics and recovery characteristics after total intravenous anaesthesia (TIVA) with xylazine-ketamine-midazolam (XKM) or dexmedetomidine-ketamine-midazolam (DKM) in horses.

Methods: Prospective, randomized experimental cross-over study. Eight horses were anaesthetized two times for two hours. After acepromazine (0.03 mg/kg bwt, IM), xylazine (0.5 mg/kg bwt, IV) or dexmedetomidine (3.5 µg/kg bwt, IV) anaesthesia was induced with ketamine (2.5 mg/kg bwt, IV) and midazolam (0.05 mg/kg bwt, IV). The TIVA was maintained with xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (7 µg/kg bwt/h) [DKM] in combination with midazolam (0.1 mg/kg bwt/h) and ketamine (3 mg/kg bwt/h). Ketamine was increased in response to positive reactions to electrical nociceptive stimulation performed every 30 min. Venous blood samples for determination of plasma concentrations of xylazine, dexmedetomidine, midazolam and ketamine were withdrawn at 5, 20, 50, 85, 120, 135, 150, 165, 180, 210, 240, 270, 300 and 1440 min. Plasma drug concentrations were determined using a high-performance liquid chromatography. Peak drug concentrations in plasma (C_{max}) were
determined directly from the data. Cardiac output (CO) [LiDCOplus-monitor] was measured before treatment (baseline), after sedation, and during anaesthesia. Twenty minutes after the end of TIVA, flumazenil (0.01 mg/kg bwt, IV) was administered. Recovery quality and duration were assessed. A two-way analysis of variance with repeated measurements was used to analyse data with an alpha of 5%.

**Results:** Mean required cumulative ketamine dose after 120 min of anaesthesia of group XKM and DKM were 3.7 ± 0.7 and 3.7 ± 0.4 mg/kg bwt/h, respectively and were not statistically different. None of the drugs reached a steady state during the two hours of TIVA. Elimination half-time after the end of the two hours infusion was 65 ± 13 min for xylazine, 46 ± 7 min for dexmedetomidine, 45 ± 11 min (XKM) and 32 ± 3 min (DKM) for midazolam and 37 ± 12 min (XKM) and 23 ± 2 min (DKM) for ketamine. With XKM CO was not different from the awake baseline at all time points. With DKM CO decreased significantly from baseline (45.6 ± 16.6 L/min) to the sedation value (35.9 ± 3.7 L/min) and remained low during the first hour of TIVA. During TIVA CO was significantly higher with XKM than with DKM. Recovery quality was good to excellent in both groups with a mean duration of 37.1 ± 16.1 and 45.5 ± 20.9 min with XKM and DKM, respectively.

**Conclusion:** Both drug combinations are suitable to maintain anaesthesia for two hours with good to excellent recovery conditions. The use of dexmedetomidine had no overt benefit compared to xylazine. Steady state was not achieved with any drug. To reach an adequate anaesthetic depth starting dose of ketamine should be elevated to 4 mg/kg bwt/h.

**Key words:** equine, xylazine, dexmedetomidine, ketamine, midazolam, flumazenil, pharmacokinetics, total intravenous anaesthesia.

### 5.2 Introduction

All volatile anaesthetics cause dose-dependent cardiopulmonary depression and hypoventilation during anaesthesia in horses (STEFFEY 2002), whereas cardiopulmonary function is well preserved during infusion of injectable drugs.
(BETTSCHART-WOLFENSBERGER et al. 2001a; BETTSCHART-WOLFENSBERGER et al. 2003; MAMA et al. 2005). An additional advantage of some injectables is the ability to reverse or antagonize drug effects if emergency situations occur and to improve recovery quality and shorten recovery time (BREHENY 1992; HOFFMAN 1993; BETTSCHART-WOLFENSBERGER et al. 1999c; BETTSCHART-WOLFENSBERGER et al. 2001a; HENKE et al. 2004; DI CONCETTO et al. 2007; UEOKA and HIKASA 2008).

Anaesthesia is produced by the combination of central nervous system (CNS) depression with hypnosis and unconsciousness, muscle relaxation and analgesia. CNS depression and analgesia can be produced by ketamine as part of total intravenous anaesthesia (MORI et al. 1971; VISser and SCHUG 2006). The routine use of ketamine based TIVA for prolonged periods of time is hampered by cumulative drug effects and prolonged time for drug elimination, which leads to prolonged recoveries with poor quality (YOUNG et al. 1993; BETTSCHART-WOLFENSBERGER et al. 1996; YAMASHITA et al. 2007). Pharmacokinetic studies showed, that during infusion plasma concentrations of the active metabolite norketamine increased progressively after two hours of anaesthesia (TAYLOR et al. 1995). This may lead to horses being tense and atactic during the recovery period (MCCARTY et al. 1990).

Analgesia and CNS depression can be enhanced with the use of α₂-adrenoceptor agonists, that additionally produce muscle relaxation (ENGLAND and CLARKE 1996). Duration and intensity of effects and side-effects depend on the type of α₂-adrenoceptor agonist, its dose and its route of administration (ENGLAND and CLARKE 1996). Xylazine is widely used as part of TIVA (MCCARTY et al. 1990; SINCLAIR and VALVERDE 2009) and has a relatively short duration of action and elimination half-life (KERR et al. 1972; GARCIA-VILLAR et al. 1981; BRYANT et al. 1991; YAMASHITA et al. 2000). Dexmedetomidine, the active D-enantiomer of medetomidine, is a highly selective α₂-adrenoceptor agonist (VIRTANEN et al. 1988; VICKERY and MAZE 1989) and its side effects last only for a very short duration.
Dexmedetomidine is rapidly redistributed and short acting which makes it suitable for infusion (BETTSCHART-WOLFENSBERGER et al. 2005).

Muscle relaxation in ketamine based TIVA can be achieved with guaifenesin (LUNA et al. 1996; MUIR et al. 2000; MCMURPHY et al. 2002) or benzodiazepines including diazepam (ROSSETTI et al. 2008), climazolam (BETTSCHART-WOLFENSBERGER et al. 1996) and midazolam (YAMASHITA et al. 2007). Availability of guaifenesin becomes more and more difficult. Further disadvantages are its potential to cause thrombophlebitis when used in high concentrations and the lack of the possibility to reverse residual effects. The imidazobenzodiazepine midazolam has some considerable advantages compared to other benzodiazepines. Midazolam is water soluble, produces less venous irritation, has a shorter distribution and elimination half-life in humans, and no active metabolite (ARENDT et al. 1983; GERECKE 1983; REVES et al. 1985). It has twice the affinity for the benzodiazepine receptor as diazepam (GERECKE 1983). The benzodiazepine antagonist flumazenil, reverses the behavioural, neurological and electrophysiological effects of benzodiazepines in man (HOFFMAN 1993) and midazolam-induced changes of electroencephalography in dogs (KEEGAN et al. 1993).

For combining different drugs for TIVA, pharmacokinetic interactions between anaesthetics are important and should be taken into account. Changes in cardiac output and hepatic blood flow induced by cardiovascularly active drugs like $\alpha_2$-adrenoceptor agonists influence drug elimination and clearance (DUTTA et al. 2000).

The aim of this study was to determine the disposition of ketamine, midazolam, xylazine or ketamine, midazolam, dexmedetomidine during and after a two hour constant rate infusion (CRI) for TIVA in horses.

5.3 Materials and Methods

5.3.1 Animals

Eight adult university-owned horses, two geldings and six mares, weighing 525 kg ± 54.4 kg (mean ± standard deviation [SD]) [450 to 580 kg] and aged 13.5 ± 6.8 years [5 to 23 years] were used. There were four standardbreds, one thoroughbred and
three warmblood horses. All horses were considered to be healthy based on physical and laboratory examination and echocardiography. The experimental protocol was approved by the Ethical Committee of the Lower Saxony State Office for Consumer Protection and Food Safety.

5.3.2 Study design
The study was carried out as a prospective, randomized experimental cross-over trial. Each horse was anaesthetized twice with at least four weeks between anaesthetic episodes.

5.3.3 Instrumentation
Before anaesthesia, each horse was weighed and body temperature, heart rate (HR), and respiratory rate (RR) were obtained. Packed cell volume (PCV), white blood cell count (WBC), total protein (TP), haemoglobin concentration (Hb) and plasma sodium concentrations (Sysmex® KX-21, Sysmex Deutschland GmbH, Norderstedt, Germany and Vitros® System, DTE II module, Ortho-Clinical Diagnostics GmbH, Neckargemünd, Germany) were determined from venous blood. The skin over the right and left jugular vein was clipped and surgically prepared. Following infiltration of the skin with mepivacaine hydrochloride (Scandicain® 2%, AstraZeneca GmbH, Wedal, Germany), a 12 gauge (G) catheter (EquiCath™ Fastflow, Braun, Tuttlingen, Germany) was placed into each jugular vein, one close to the superior thoracic aperture for drug administration and one for blood collection. The skin over the right transverse facial artery was shaved, cleaned and desensitized with a transdermal anaesthetic cream containing lidocaine and prilocaine (EMLA®, AstraZenca GmbH, Wedel, Germany). For cardiac output determination, a 20 G catheter (Venocan™ IV Catheter, Kruuse, Langeskov, Denmark) was placed in the right transverse facial artery and connected to a pre-calibrated disposable pressure transducer (DTXPlus® Pressure Transducer System, Becton Dickinson GmbH, Heidelberg, Germany), set at the level of the right atrium.

5.3.4 Drug combinations
All horses were premedicated with acepromazine (0.03 mg/kg bwt IM) [Vetranquil® 1%, Albrecht GmbH, Aulendorf, Germany] 15 min before sedation with the tested α2-
adrenoceptor agonist. Two drug combinations were used for TIVA. Combination 1 (XKM) consisted of sedation with 0.5 mg/kg bwt xylazine IV followed by ketamine (2.5 mg/kg bwt IV) [Narketan® 100mg/ml, Vétoquinol GmbH, Ravensburg, Germany] and midazolam (0.05 mg/kg bwt IV) [Midazolam-ratiopharm® 15mg/3ml, ratiopharm GmbH, Ulm, Germany] for induction of anaesthesia. Anaesthesia was maintained by infusion of ketamine (starting dose of 3 mg/kg bwt/h IV) midazolam (0.1 mg/kg bwt/h IV) and xylazine (1.0 mg/kg bwt/h IV). Combination 2 (DKM) consisted of IV administration of 3.5 µg/kg bwt dexmedetomidine for sedation followed by ketamine (2.5 mg/kg bwt IV) and midazolam (0.05 mg/kg bwt IV) for induction of anaesthesia. Anaesthesia was maintained with ketamine (starting dose of 3 mg/kg bwt/h IV), midazolam (0.1 mg/kg bwt/h IV) and dexmedetomidine at a dose of 7 µg/kg bwt/h IV in group DKM. For midazolam antagonism, all horses received flumazenil (0.01 mg/kg bwt IV) [Flumazenil HEXAL® 0,1 mg/ml Injektionslösung, HEXAL AG, Holzkirchen, Germany].

5.3.5 Experimental protocol

After induction of anaesthesia, an endotracheal tube was placed and horses were hoisted on a padded surgery table in left lateral recumbency. Five minutes after induction, TIVA was started (anaesthesia time zero). Drugs were administered with three separate syringe pumps (perfusor® compact, B. Braun Melsungen AG, Melsungen, Germany) to maintain anaesthesia. The animals were breathing spontaneously an air-oxygen mixture with an inspired oxygen fraction (FiO$_2$) of 0.5 - 0.6 from a large animal circle breathing system (Vet.-Tech. Model JAVC-2000 J.D. Medical Distributing Company, Phoenix, USA). Ringer’s lactate (Ringer Ecobag® click, B.Braun Melsungen AG, Melsungen, Germany) was infused at a rate of 5 ml/kg bwt/h IV. Electrical stimulation was performed first 30 min after induction and thereafter every 30 min. Ketamine infusion rate was increased by 0.5 mg/kg bwt/h in case of positive reaction to electrical stimulation or spontaneous movement. Thiopental (1 mg/kg bwt IV) [Trapanal®, Nycomed Deutschland GmbH, Konstanz, Germany] was given if the horse was trying to rise.
After 120 min of anaesthesia the infusion was discontinued, the total dose of anaesthetic drugs recorded and horses hoisted in a padded recovery box. Ten millilitres of phenylephrine [Phenylephrine 0.15%, Löwenapotheke, Germany] were instilled into the nares to decongest the nasal cavities. During recovery, oxygen was insufflated at 15 L/min initially through the endotracheal tube, after extubation through the ventral nasal meatus. All horses received flumazenil (0.01 mg/kg bwt IV) [Flumazenil HEXAL® 0.1 mg/ml Injektionslösung, HEXAL AG, Holzkirchen, Germany] 20 min after the end of TIVA. Duration of lateral and sternal recumbency and total time to standing position were recorded. Recovery quality was scored based on a previously published 100-point scoring system form Clark-Price et al. with 11 categories and a possible total score range of 11 to 100. A score of 11 would be an ideal recovery, whereas a score of 100 would be the most uncoordinated, fractions recovery. (CLARK-PRICE et al. 2008).

5.3.6 Measurements
Cardiac output measurements were performed using the lithium dilution technique [LiDCOplus® Hemodynamic Monitor, LiDCO Ltd, London, UK] at 30, 60, 90 and 120 min (HALLOWELL u. CORLEY 2005). A special software was installed to accommodate the LiDCOplus to measurements in large animals (LiDCOplus ver. V4 Vet Configuration). Blood haemoglobin and plasma sodium concentration were entered into the LiDCOplus monitor. A bolus of 2.25 millimol (mmol) of lithium chloride (LiCl) was delivered manually through the drug catheter into the jugular vein. The LiCl was injected five s after initiating the measurement, to allow the 12 seconds (sec) of stable baseline required for accurate CO calculation (HALLOWELL and CORLEY 2005). For the detection of the LiCl by the LiDCO sensor, arterial blood was withdrawn from the transverse facial artery by the LiDCO Flow Regulator.

5.3.7 Blood sampling
Blood samples for drug analysis were collected from a separate drug sampling catheter in the jugular vein before and 2 min after sedation as well as 5, 20, 50, 85, 120, 135, 150, 165, 180, 210, 240, 270, 300 and 1440 min after induction of anaesthesia (start of TIVA). Ethylenediaminetetraacetate (EDTA) was used as the
anticoagulant. The blood was immediately centrifuged at 850G for six min and plasma was frozen at -80°C until analysis.

5.3.8 Drug analysis

5.3.8.1 Dexmedetomidine

Dexmedetomidine concentrations in plasma were determined with a fully validated method based on reverse-phase high-performance liquid chromatography with tandem mass spectrometric detection [HPLC-MS/MS; Shimadzu Prominence HPLC connected to an AB Sciex API4000 mass spectrometer] as previously described (IIROLA et al. 2011). The mobile phase was 0.1 % formic acid in a mixture of 1:1:1 (v/v/v) methanol/acetonitrile/water. The lower limit of quantification was 0.05 ng/ml plasma. The inter-assay precision (c.v. %) for quality control samples in the study sample batches was within 4 % in the relevant concentration range. Samples were analysed at the Department of Pharmacology, Drug Development and Therapeutics, University of Turku and Unit of Clinical Pharmacology, TYKSLAB, Turku, Finland.

5.3.8.2 Xylazine, ketamine and midazolam

Xylazine, ketamine and midazolam concentrations in plasma were determined at 215 nanometre (nm) with a HPLC system using the external standard method [Pump (126 Solvent Module), autosampler (507) and detector (168), Beckman (Munich, Germany); Column heater: 40°C, Spark Holland (Emmen, Netherlands); HPLC-Column: LiChrospher 100, CN (5 µm), Merck (Darmstadt, Germany); 25 cm, guard column: LiChrospher 100 CN (5 µm), Merck (Darmstadt, Germany); Software: 24 Karat 5.0, (Beckmann, Munich, Germany)]. The mobile phase was 60 % acetonitrile and 40 % phosphate buffer (pH 5.5; 9.12 g KH₂PO₄ and 9.24 g Na₂HPO₄ x 2H₂O in 1l purified water). The lower limit of quantification was 50 ng/ml plasma for all compounds. The inter-assay precision (c.v. %) for quality control samples in the study sample batches was < 20% in the relevant concentration range.

5.3.8.3 Pharmacokinetic calculations

The peak drug concentrations in plasma (C(max)) and the corresponding time points (t(max)) were determined directly from the data. For each horse, the terminal linear
phase of the drug plasma concentration-time curve was visually identified. The elimination half-time \((t_{1/2})\) was then calculated from the following equation: \(t_{1/2} = \frac{\ln 2}{k_e}\). The pharmacokinetic data were analysed with the WinNonlin pharmacokinetic program (version 5.3; Pharsight, Mountain View, CA, USA) using the non compartmental approach.

5.3.9 Statistical analysis
Goodness of fit for normal distribution of model residuals of parameters was assumed by visual assessment of normal probability plots and the Shapiro-Wilk test. Characteristics of recovery from anaesthesia were neither normally nor log-normally distributed. For these data nonparametric methods were used. Wilcoxon signed rank test for not normally distributed matched pairs was used to compare characteristics of recovery from anaesthesia between groups. Data were presented as arithmetic mean and SD, except half-times, which were expressed as harmonic mean and jack-knife SD (LAM et al. 1985). A Pearson chi-square test was used to evaluate group differences in thiopental use and for correlation of ketamine infusion rate and corresponding plasma concentration.

Analyses were carried out with the statistical software SAS, version 9.2 (SAS Institute, Cary, NC). Values of \(p < 0.05\) were considered significant.

5.4 Results

5.4.1 Anaesthesia
Mean required cumulative ketamine dose after 120 min of anaesthesia of group XKM and DKM were 3.7 ± 0.7 mg/kg bwt/h and 3.7 ± 0.4 mg/kg bwt/h, respectively and were not statistically different. There was no statistically significant difference between the numbers of positive reactions to noxious stimulation, but spontaneous movements occurred more often in group XKM (seven times within group XKM and two times within group DKM). The character of purposeful movement and its intensity was more severe in group XKM which resulted in significantly \((p=0.02)\) more thiopental injections in group XKM (three horses 1x, two horses 2x, one horse 3x) than in group DKM (1x). Recovery times of groups XKM and DKM were 37.1 ± 16.1 and 45.5 ± 20.9 min, respectively with a tendency to be shorter in group DKM.
(p=0.057). Recovery scores of groups XKM and DKM were 32.5 ± 14.5 and 20.9 ± 10.9, respectively ([p=0.0643] (Table 3).

5.4.2 Cardiorespiratory variables
Cardiac output was significantly higher in XKM during TIVA (Table 3). Further cardiovascular and respiratory variables are reported elsewhere.

5.4.3 Pharmacokinetics
Steady state drug concentrations were not achieved during the infusion period (Figure 6 to Figure 9). Maximum plasma concentration and corresponding time during anaesthesia and elimination half-time is shown in Table 9. For determination of $t_{1/2}$ three horses were excluded for xylazine and midazolam (XKM and DKM) and four horses were excluded for ketamine (XKM). The correlation between infusion rate of ketamine and its plasma concentration during anaesthesia was low ($r=0.37$) [Figure 10]. Overall, mean ketamine plasma concentrations were low (Figure 6). In one horse of group XKM, the plasma ketamine concentration stayed below 0.5 µg/ml, although ketamine infusion was elevated continuously reaching a dose of 5 mg/kg bwt/h for the last 30 min of anaesthesia. This horse reacted three times to nociceptive stimulation with purposeful movements and required thiopental. The same horse also had values below 0.5 µg/ml of ketamine during the first 85 min in group DKM, but here the last value during anaesthesia increased to 1.34 µg/ml and the horse did not need any top up.

5.5 Discussion
The cardiac function was maintained within acceptable limits in all horses with both protocols. The determination of cardiac output was used as a global indicator of central perfusion. Hepatic blood flow, and therefore cardiac output influences pharmacokinetics of drugs that are extensively metabolized by the liver (DUTTA et al. 2000). Whereas CO increased over time, reaching higher values than in the awake state in group XKM, CO was lower than in the awake horses in the first hour of anaesthesia in group DKM. This difference may have influenced elimination of drugs and recovery time. Although all horses recovered in a coordinated manner, animals in group DKM did not recover as promptly as in group XKM. The infusion rate of
dexmedetomidine was determined as equisedative to 1 mg/kg bwt/h of xylazine in previous sedation trials (published elsewhere). However, the cardiovascular effects of these equisedative doses were not compared in the awake state.

Elimination half-lives of dexmedetomidine in horses are reported to be shorter than those of xylazine. For dexmedetomidine, elimination half-life was 19.8 ± 9.63 min in young ponies and 28.96 ± 7.61 min in mature horses sedated with a bolus of 3.5 µg/kg IV (BETTSCHART-WOLFENSBERGER et al. 2005). For xylazine, elimination half-life was 49.51 min after bolus administration of 0.6 mg/kg bwt IV (GARCIA-VILLAR et al. 1981). The elimination half-life after a single drug bolus is of little value in characterizing disposition of intravenous anaesthetic drugs during infusion periods relevant to anaesthesia, but the context-sensitive half-time has been proposed as a more useful measure of the pharmacokinetic offset of intravenous anaesthetics (HUGHES et al. 1992). Despite the fact that post-infusion kinetics are better described by context-sensitive half-time than by elimination half-life, the relationship between drug concentration and drug effect is not a simple linear response. If dosing has been excessive, a decline in drug concentration of more than 50% is required before adequate recovery will occur (KAPILA et al. 1995). When an anaesthetic drug is administered as an intravenous bolus, there is a rapid increase in plasma concentration, followed by rapid distribution and redistribution of the drug throughout the body and recovery is mainly independent of metabolism and elimination (MCMURPHY 2005). Distribution and redistribution of a drug already occurs during infusion, so it is not surprising, that elimination half-time is longer after infusion of the drug like in our study. There are no reports of $t_{1/2}$ for xylazine and dexmedetomidine after infusion in horses. In our study elimination of dexmedetomidine was more prolonged than that of xylazine compared to values for elimination half-life after bolus administration. The prolonged elimination half-time is possibly related to the lower CO in group DKM. In a previous study, a third group for TIVA was included with a lower dose of dexmedetomidine (group DKM1: 7 µg/kg bwt/h of dexmedetomidine, a starting dose of 3 mg/kg bwt/h and 0.1 mg/kg bwt/h of midazolam) and resulted in less cardiac depression. An increase of dose of α2-adrenoceptor agonists up to the maximal effects resulted in a longer depression of CO and a further increase of

The infusion rate of xylazine and midazolam was similar to the rates used in other studies for TIVA (GREENE et al. 1986; YAMASHITA et al. 2007). The infusion rate of ketamine was kept low at the beginning of anaesthesia, because the study aimed to determine the dose of ketamine in combination with a fixed dose of xylazine or dexmedetomidine and midazolam, which is necessary to provide surgical anaesthesia. At the beginning, the level of anaesthesia was too light in the majority of horses to provide surgical anaesthesia because almost all horses reacted to nociceptive electrical stimulation and ketamine infusion rate had to be elevated stepwise. These clinical observations are supported by the plasma concentrations of ketamine during anaesthesia. At the beginning of anaesthesia, in individual animals plasma concentrations were below 1 µg/ml in both groups. In adult horses mean plasma concentration of ketamine corresponding to recovery from anaesthesia was estimated from the least-squares line and was at least 1.01 ± 0.41 µg/ml (KAKA et al. 1979). After TIVA with propofol and ketamine, plasma ketamine concentrations were 0.70 to 1.08 µg/ml at the time when the ponies assumed sternal recumbency (NOLAN et al. 1996). Another study reported plasma ketamine concentrations of 1.01 ± 0.41 µg/ml at the time of first movement and 0.87 ± 0.14 µg/ml at the time of standing up after a TIVA with detomidine, ketamine and guaifenesin (TAYLOR et al. 1995). Ketamine alone at a dose of 9.0 mg/kg bwt/h was not sufficient to prevent response to noxious stimulation (50 V, 5 Hz, 10 ms) via insulated needle electrodes to the buccal mucosa, but lower doses of ketamine in combination with xylazine did, which indicates that not only the plasma concentration of ketamine is responsible for immobility (MAMA et al. 2005). Despite the fact that plasma concentrations of ketamine were low compared with other studies, no horse did react to electrical stimulation at the end of anaesthesia although ketamine concentrations sometimes stayed below 1 µg/ml plasma. The inter-individual variability of plasma ketamine concentration where horses did not react to noxious stimulation was high and the correlation between infusion rate and plasma concentrations was weak. A reason for the low correlation and the high intra- and inter-individual variability might be the
inhomogeneity of the type of horses included into the study. In addition to different breeds, differences in age ranged from 5 to 23 years and body weight ranged from 480 to 600 kg. Although the inhomogeneous configuration of groups complicates analysis of pharmacokinetic data, it reflects more reliably the daily routine of equine anaesthesia. Geriatric individuals show a lower clearance that may lead to a longer duration of action and longer half-lives (LEES et al. 1985; BETTSCHART-WOLFENSBERGER et al. 2005). In dogs, bodyweight, age and breed influenced clearance and herewith elimination plasma half-life (COX et al. 2011). Advanced age has been associated with decreased hepatic clearance of many drugs in humans and has been attributed for example to factors such as decreased activity of certain cytochrome P450 enzymes, decreased liver size and decreased hepatic blood flow. Also, the influence of age is associated with conditions such as disease, frailty and stress. Further, the administered drugs, especially the α-adrenoceptor agonists, may have effects, which are mainly dose-dependent, on regional blood flow distribution like hepatic blood flow and therefore elimination clearance.

It is also necessary to point out, that plasma concentrations do not stand for the effect site, which is mainly the CNS for anaesthetics. Pharmacokinetic parameters (e.g. half-life or context sensitive half-time) of different drugs do not predict the relative rates of decrease in effect site concentrations neither after an intravenous bolus nor after a continuous infusion (SHAFER and VARVEL 1991). The high intra-individual changes in some horses could perhaps be explored in detail with a more frequently performed sampling of blood to avoid large gaps within the concentration-time curve.

The calculated elimination half-time of ketamine in both groups can be compared to those reported elsewhere for ketamine disposition in horses. After bolus administration of ketamine an elimination half-life of 42 and 65.8 ± 10.9 min for ketamine using a two-compartmental model is reported (KAKA et al. 1979; WATERMAN et al. 1987). However, after TIVA with ketamine and climazolam for 120 min ketamine elimination half-time was 282 ± 32.6 min (BETTSCHART-WOLFENSBERGER et al. 1996) and after 60 min TIVA with propofol and ketamine
$t_{1/2}$ was 69 ± 8 min (NOLAN et al. 1996). In our study $t_{1/2}$ was calculated as 37 ± 12 and 23 ± 2 min for XKM and DKM, which is clearly lower than elimination half-life described in the literature (all three studies used a non-compartmental model). It is also important to realize, that values derived for the context-sensitive half-time are a result of computer simulations that use the identical pharmacokinetic computations as used in target-controlled infusion devices to predict drug concentration from drug dosing schemes. Only if the pharmacokinetic parameters describe the disposition of the drug accurately, the context sensitive half-time is correct. HUGHES et al. (1992) showed for six intravenous anaesthetics, that the time required for the plasma drug concentration to decline by 50 % after terminating prolonged infusions were markedly different from their respective elimination half-lives. The study by KAPILA et al. (1995) attempted to validate context-sensitive half-times and showed that measured drug concentration of remifentanil and alfentanil after a three hour infusion were successfully predicted by the context-sensitive half-times but were not predicted by the elimination half-lives. In our study, the difference of elimination half-time of both TIVA protocols compared to the literature might be related to cardiac index, that was decreased to 65 % in the study of BETTSCHART-WOLFENSBERGER et al. (1996), whereas it decreased to 78 % with DKM and increased to 130 % with XKM in the current study. Another reason might be the lower limit of detection which was 0.05 µg/ml in our study and 0.01 µg/ml in the studies mentioned above. In our study, plasma concentration decreased relatively fast below lower limit of detection and hence residual decrease to 0.01 µg/ml, like in the other studies, was not included in determination of elimination half-time. In the current study, for determination of $t_{1/2}$ only four horses could be included with XKM, and eight horses with DKM, because plasma concentration decreased very fast below lower limit of detection and hence, influenced determination of elimination half-time.

For midazolam, there are no values published for elimination half-life or half-time in horses. In humans, the elimination half-life ranged from 1.7 to 3.5 h in humans (ALLONEN et al. 1981; HEIZMANN et al. 1983; GREENBLATT et al. 1984; MISAKA et al. 2010). For diazepam, another benzodiazepine, pharmacokinetic data for use in horses exist. Its elimination half-life after single intravenous injection is reported to be
in the range of 6.94 to 13.2 h in horses (MUIR et al. 1982; SHINI et al. 1997). In our study, elimination half-time of midazolam was 45 ± 11 min (XKM) and 33 ± 3 min (DKM), which is clearly shorter than elimination half-time of diazepam. The knowledge of elimination half-time of midazolam is important to improve recovery quality in particular, because we used a specifically acting benzodiazepine antagonist flumazenil in our study to enhance recovery quality. The elimination characteristics of both, agonist and antagonist are important to avoid ataxic recoveries and resedation. Elimination half-time of the benzodiazepine agonist should not overlast that of the antagonist, which favours the use of the short acting midazolam. For horses, no pharmacokinetic data of flumazenil exist. In humans, elimination half-life ranges from 40 to 80 min (OLKKOLA and AHONEN 2008). Nevertheless, a single intravenous dose was usually sufficient to attain and maintain for about two hours the desired level of consciousness after general anaesthesia in men (BROGDEN and GOA 1988). Clinically, we had no signs of resedation and we had no secondary peak in concentration-time profile of ketamine and midazolam after administration of flumazenil like observed by BETTSCHART-WOLFENSBERGER et al. (1996) with climazolam and sarmazenil. This is probably the benefit of the shorter elimination characteristics of midazolam compared with other benzodiazepines. We did not measure the plasma concentration of flumazenil, but clinically we had no signs of inadequate reversal or resedation.

With none of the drugs, a steady state was achieved. For ketamine, it is not remarkable because we elevated its dose depending upon anaesthetic depth and had no constant infusion rate from the beginning of anaesthesia. Although we had a constant infusion rate for dexmedetomidine or xylazine and midazolam, plasma concentration showed high intra- and inter-individual changes. Changes in anaesthetic depth might have influenced metabolism and regional blood flow, especially if movement occurred after nociceptive electrical stimulation. With dexmedetomidine and midazolam, no peak in plasma concentration time curve was visible after bolus administration. Due to the short elimination half-life after bolus administration of dexmedetomidine, the peak concentration might not be identified because of the relatively long blood sampling intervals. For midazolam, the dose of
the bolus might be too low to be detected by the method used. Beside the missing peak after bolus administration, concentration time curves showed a continuous increase over time. A higher loading dose might be helpful to reach a higher level of plasma concentrations faster. Clinically, all horse showed a sufficient muscle relaxation, but an elevation of the loading dose would perhaps result in less movement at the beginning of anaesthesia.

In conclusion, the aim to produce a surgical anaesthesia while using low doses of ketamine was achieved with the combination of both $\alpha_2$-adrenoceptor agonists and midazolam. Cardiopulmonary function and recovery quality was excellent, especially with XKM. The tendency of difference between positive reaction to noxious stimulation and the significant higher frequency of applying thiopental as top up in group XKM may indicate, that dexmedetomidine is more analgesic than xylazine. The characteristics for elimination support the clinically shorter duration of recovery with XKM. The clinical knowledge from this study could be used to perform a study with a fixed anaesthetic protocol that provides surgical anaesthesia from the beginning. More homogenous horses could help to elaborate pharmacokinetics of dexmedetomidine or xylazine, ketamine and midazolam used for TIVA as well as to evaluate reasons for the low correlation between ketamine infusion and plasma concentration and for the absence of steady state for all drugs.
### 5.6 Tables and Figures

**Table 7**: Mean recovery time and recovery quality in horses after anaesthesia with a CRI of xylazine (1 mg/kg bwt/h), [XKM] or dexmedetomidine (7 µg/kg bwt/h) [DKM], ketamine and midazolam (0.1 mg/kg bwt/h IV).

<table>
<thead>
<tr>
<th></th>
<th>XKM</th>
<th>DKM</th>
</tr>
</thead>
<tbody>
<tr>
<td>recovery time (min)</td>
<td>37.1 ± 16.1</td>
<td>45.5 ± 20.9</td>
</tr>
<tr>
<td>recovery quality (score points)</td>
<td>32.5 ± 14.5</td>
<td>20.9 ± 10.9</td>
</tr>
<tr>
<td>ketamine dose (mg/kg bwt/h)</td>
<td>3.7 ± 0.7</td>
<td>3.7 ± 0.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

**Table 8**: Cardiac output (CO) before treatment (baseline), immediately after sedation (sedation) and in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h), [XKM] or dexmedetomidine (7 µg/kg bwt/h) [DKM], ketamine and midazolam (0.1 mg/kg bwt/h IV).

<table>
<thead>
<tr>
<th>time</th>
<th>group</th>
<th>CO (L)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>XKM</td>
<td>47 ± 25</td>
<td>DKM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46 ± 17</td>
<td></td>
</tr>
<tr>
<td>sedation</td>
<td>XKM</td>
<td>32 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DKM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33 ± 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>XKM</td>
<td>47 ± 16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>DKM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>32 ± 9&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>XKM</td>
<td>56 ± 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DKM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>33 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>XKM</td>
<td>61 ± 25</td>
<td>DKM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 ± 10</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>XKM</td>
<td>61 ± 18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>DKM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>36 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD. <sup>a</sup> Significantly (p < 0.05) different than baseline value. <sup>b</sup> Significant (p < 0.05) difference between group XKM and DKM.
Table 9: Pharmacokinetic parameters of eight adult horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (7µg/kg bwt/h) [DKM], ketamine (3.0 mg/kg bwt/h, starting dose) and midazolam (0.1 mg/kg bwt/h).

<table>
<thead>
<tr>
<th>horse</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>t&lt;sub&gt;max&lt;/sub&gt;</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Xyl µg/ml</td>
<td>Dex ng/ml</td>
<td>Ket µg/ml</td>
</tr>
<tr>
<td>1</td>
<td>0.65</td>
<td>3.59</td>
<td>1.47</td>
</tr>
<tr>
<td>2</td>
<td>1.28</td>
<td>3.30</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>/</td>
<td>9.20</td>
<td>/</td>
</tr>
<tr>
<td>4</td>
<td>0.74</td>
<td>3.74</td>
<td>0.46</td>
</tr>
<tr>
<td>5</td>
<td>0.77</td>
<td>7.72</td>
<td>1.67</td>
</tr>
<tr>
<td>6</td>
<td>2.12</td>
<td>2.97</td>
<td>1.89</td>
</tr>
<tr>
<td>7</td>
<td>1.89</td>
<td>3.36</td>
<td>4.60</td>
</tr>
<tr>
<td>8</td>
<td>1.53</td>
<td>2.69</td>
<td>2.72</td>
</tr>
<tr>
<td>mean</td>
<td>1.28</td>
<td>4.57</td>
<td>1.92</td>
</tr>
</tbody>
</table>

C<sub>max</sub> peak plasma concentration, t<sub>max</sub> time corresponding to C<sub>max</sub>, t<sub>1/2</sub> elimination half-time (reported as harmonic mean and jack-knife variance)
Figure 6: Mean ± SD of plasma concentration [µg/ml plasma] and constant rate infusion [mg/kg bwt/h] of ketamine in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (7 µg/kg bwt/h) [DKM], ketamine and midazolam.
Figure 7: Mean ± SD of plasma concentration [µg/ml plasma] of xylazine in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h) [XKM], ketamine and midazolam.
Figure 8: Mean ± SD of plasma concentration [ng/ml plasma] of dexmedetomidine in horses anaesthetized with a CRI dexmedetomidine (7 µg/kg bwt/h) [DKM], ketamine and midazolam.
Figure 9: Mean ± SD of plasma concentration [µg/ml plasma] of midazolam in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (7 µg/kg bwt/h) [DKM], ketamine and midazolam.
Figure 10: Correlation between infusion rate [mg/kg bwt/h] and plasma concentration [µg/ml] of ketamine. The linear fit is shown as black line, and the upper and lower 95% confidence limits are indicated as dashed lines.

REFERENCES see page 115 and the following
6 General discussion

6.1 Materials and methods
The aim of the thesis was to compare the effects of dexmedetomidine and xylazine on sedation and as TIVA combined with ketamine and midazolam on cardiopulmonary function and recovery in horses.

6.1.1 Study design
The study was carried out as a prospective, randomized experimental cross-over trial. One advantage of this study design is that we excluded individual characteristics as reason for changes between protocols. The succession which treatment a horse achieved first was at random. Therefore, the fact that horses became familiar with procedures before induction and even during recovery was not related to a special treatment. The third trial during pretrials was not carried out at random, because the decision to evaluate a higher dose of dexmedetomidine was made as a result of findings of the two previous trials. Therefore, the effect of horses getting familiar to procedure cannot be excluded. The weight and especially the age of the horses involved in this study showed a relatively large standard deviation. Horses of three different breeds were involved. Because of the chosen study design these differences did not influence results between groups but the individual results within one group. Especially during thermal stimulation in the awake horses, the different behaviour of the trotters compared with the warmblood horses was obvious. Pharmacokinetic data showed also a high intra- and interindividual variation, which might be related to the inhomogeneity of the group of horses.

6.1.2 Preliminary trial
During the preliminary sedation trial depth of sedation with xylazine was judged by a standard method with the NGD being the main factor for the assessment of the sedation depth. This procedure was used in several other studies (KAMERLING et al. 1988; BRYANT et al. 1991; BETTSCHART-WOLFENSBERGER et al. 1999b). The response to visual and acoustical stimuli can also be used to describe the degree of sedation (HOPSTER et al. 2008). The analgesic effect of different treatments was judged by application of thermal stimuli to the area over the horse’s
right nostril. This implicates transmission of the noxious stimulus via the sensory infraorbitalis nerve, leaving out the spinal route of nociceptive pathways. Thermal stimulation at the horse’s withers to produce a skin twitch reflex would include spinal transmission (ROBERTSON et al. 2005; SANCHEZ et al. 2007; ELFENBEIN et al. 2009). However, a recent study in our group has shown that analgesic effects of drugs can equally be detected with stimulation at the nostrils and the withers (DISS POLLER, in preparation). With the head down position head oedema developed in some horses and they started to transpire especially beyond the heating element, which might have influenced sensibility towards thermal stimulation and thermal conduction. Some horses were excited while standing in the stock, especially before sedation during evaluation of the baseline values. Hence, excitement might be a reason why some horses did not visibly react to thermal stimulation during baseline determination. These unsuccessful stimulations were excluded for determination of baseline TT and might have influenced the means. A possibility to avoid this problem and to improve the test conditions might be testing free moving horses in their daily surroundings. However, in the current study set up it was the idea to determine the NGD concurrently.

The bolus of 0.5 mg/kg bwt followed by a CRI of 1 mg/kg bwt/h xylazine IV was set as reference concerning depth of sedation and not the often cited higher bolus of 1 or 1.1 mg/kg bwt IV of xylazine for premedication in horses (GREENE et al. 1986; YOUNG et al. 1993; BETTSCHART-WOLFENSBERGER et al. 1996; MUIR et al. 2000; OKU et al. 2005; REZENDE et al. 2010), because clinical experience with our population of horses showed, that they became very atactic and some collapsed with this bolus. For D2, the application of boli of dexmedetomidine to reach the same percentual decrease of head drop than before with xylazine might not have the same effect as the resulting constant rate infusion. Bolus administration might result in a serrated plasma concentration time curve with peaks after bolus whereas a constant rate infusion does not. This may influence degree of head drop and reaction to thermal threshold. The possibility that stimulation occurred shortly after bolus administration may result in higher thermal thresholds.
6.1.3 Anaesthetic protocol

Preanaesthetic sedation with α₂-adrenoceptor agonists and induction with ketamine and midazolam were commonly used in horses because of a low incidence of adverse reactions (TAYLOR and CLARK 2007) and the comparatively low risk of death (JOHNSTON et al. 1995).

For anaesthesia, xylazine or dexmedetomidine, midazolam and ketamine was given separately with three syringe pumps in the current study which enables individual adjustment of the ketamine dose according to anaesthetic depth compared to an application via a combined infusion containing all drugs, like a triple drip.

In our study, TIVA contained xylazine or dexmedetomidine, ketamine and midazolam. The dose of xylazine and dexmedetomidine for sedation and constant rate infusion were chosen based on pretrials. Anaesthesia induction with ketamine and midazolam followed a standard procedure, containing 2 – 2.5 mg/kg bwt, IV of ketamine and 0.04 – 0.2 mg/kg bwt, IV of midazolam. This protocol is characterized by an excellent, rapid and excitement-free quality of induction with good muscle relaxation (LUNA et al. 1997; KUSHIRO et al. 2005; UMAR et al. 2007; VALVERDE et al. 2007; YAMASHITA et al. 2007). Doses of ketamine were within the lower range previously described for TIVA (see above) and dose of midazolam was a little higher than described by Yamashita et al (2007), who used 0.08 mg/kg bwt/h for TIVA, but they did not antagonize it for recovery.

6.1.4 Measurement methods

During anaesthesia the following cardiovascular and respiratory variables were measured continuously and recorded every 10 min: RR, HR, arterial blood pressure, inspiratory and expiratory oxygen fraction, expiratory carbon dioxide fraction and body temperature. Arterial blood-gas and pH analysis and cardiac output measurement by lithium dilution technique were performed every 30 min. Additionally, CO was measured continuously by pulse contour analysis after the first calibration at 30 min. This offered the possibility to include short-term changes into analysis. Arterial blood gas analysis was performed immediately, so that results were not influenced by long storage or contamination with air. Lithium dilution
measurement is an established method (LINTON et al. 2000; CORLEY et al. 2003; HALLOWELL and CORLEY 2005) and eliminates the need for right heart catheterization that is necessary to perform for example thermodilution. The length of time the LiCl needs to reach the sensor is dependent upon the anatomical location of the arterial catheter. We always catheterized the same artery, which avoided the need to adapt the measurement method to this difference. Systemic vascular resistance was calculated using a published formula including MAP and CO (EDNER et al. 2005). The right atrial pressure was not included into the formula because we did not measure it, but it would have precised values (SCHAUVLIEGE et al. 2008). As we only compared changes over time, all measurements had the same inaccuracy and had thus no influence to our results.

6.1.5 Blood sampling for pharmacokinetic analysis

Our results of determination of plasma concentrations showed high individual variations and a fast decline of plasma concentrations of measured drugs below the lower limit of detection. Consequently, it was not possible to determine elimination kinetics of some horses. The possibility of cross contamination and reason for the high variation of plasma concentration was nearly avoided because blood samples for kinetic analyses were collected from a separate drug sampling catheter of the jugular vein. Before sampling, 4 ml of blood were withdrawn; then 9 ml of blood were collected into EDTA-containing vacutainers (Vacuette EDTA K3, Greiner Bio-One GmbH, Essen, Deutschland). This catheter was never used for drug administration. For each sample a new syringe and cannula were used. After blood collecting, the catheter was flushed with heparinized saline solution.

During the first hour after stopping CRI sampling of blood was performed in 15 minutes intervals. Depending on the time when horses tried to stand up and how coordinated they were directly after standing, blood sampling had to be delayed because it was too dangerous to go inside the recovery box. A more frequent sampling of blood after stopping CRI would be useful to evaluate the pharmacokinetics of elimination because at least three values were necessary to determine the elimination phase.
The inter-assay precision was different between methods to determine dexmedetomidine concentration and concentration of the other used drugs. For ketamine, midazolam and xylazine, the precision was relatively low and the lower level of detection was relatively high, especially for ketamine. The lower limit of detection for ketamine found in the literature was 0.01 µg/ml plasma with an interassay coefficient of variation between 1.3 to 8.7 % (WATERMAN et al. 1987; TAYLOR et al. 1995; BETTSCHART-WOLFENSBERGER et al. 1996; NOLAN et al. 1996). The lower limit of detection for midazolam in literature was 0.1 ng/ml plasma with an intra-assay coefficient of variation of <6.3% in humans (MISAKA et al. 2010). The lower limit of detection for xylazine is reported as 0.01 µg/ml plasma, compared to 0.05 µg/ml in our study. For dexmedetomidine, the lower limit of detection reported in the literature ranged from 0.01 to 0.05 ng/ml plasma in humans (DYCK et al. 1993; DUTTA et al. 2000; IIROLA et al. 2011) and was 0.05 ng/ml in horses (BETTSCHART-WOLFENSBERGER et al. 2005), which was the same as in our study. The interassay coefficient of variation ranged between 7.5 and 11.3% (DYCK et al. 1993; DUTTA et al. 2000; IIROLA et al. 2011).

6.1.6 Postoperative monitoring

All horses received flumazenil (0.01 mg/kg bwt IV) [Flumazenil HEXAL® 0,1 mg/ml Injektionslösung, HEXAL AG, Holzkirchen, Germany] 20 min after the end of TIVA or at the moment where horses attempted to rise. Some horses made attempts to leave lateral recumbency earlier than 20 min after the end of TIVA. If possible, horses were forced to stay in lateral recumbency to enable administration of flumazenil at the exact time, which was possible in all but one horse that got flumazenil 16 min after end of anaesthesia. Some of these horses reached the standing position directly without having a sternal phase. Through our interference recovery quality as well as recovery time might be advanced. The recovery period was scored based on a previously published 100-point scoring system from CLARK-PRICE et al. (2008) directly by one investigator. To make the assessment of the recovery period more objective a second person that is not involved into the study could additionally estimate the score for recovery period via videotape without having any knowledge of treatment and how the horse behaved during previous trials. On the other hand, the
scoring system is very detailed with fixed categories and fixed points so that the possibility for individual subjective validation is low.

6.2 Results
As we wanted to compare cardiopulmonary function and recovery parameters of three different drug combinations for TIVA in horses, we initially evaluated the equipotent dose of xylazine and dexmedetomidine. The extent and duration of head drop had been the main criterion to judge the degree of sedation in horses (Figure 11, appendix), which is an accepted criterion for \( \alpha_2 \)-adrenoceptor agonists induced sedation in other studies (KAMERLING et al. 1988; BRYANT et al. 1991; BETTSCHART-WOLFENSBERGER et al. 1999b). The bolus of 0.5 mg/kg bwt followed by a CRI of 1 mg/kg bwt/h xylazine IV reduced NGD by 73% five minutes after bolus was given. Accordingly, the bolus of 3.5 µg/kg bwt of dexmedetomidine resulted only in a reduction of 43% and 64% in group D1 and D2. A reason for the difference between head drop after the same dexmedetomidine bolus in group D1 and D2 might be the fact that horses were getting used to the set up for group D2, which was always performed during the third trial whereas the succession of group X and D1 was at random.

In the literature, a dose of 3.5 µg/kg bwt of dexmedetomidine was judged to be equisedative to 7 µg/kg bwt of medetomidine or 1 mg/kg bwt of xylazine in ponies (BETTSCHART-WOLFENSBERGER et al. 2005). Another study suggested that equipotent sedative doses in horses are 7.7 µg/kg bwt of medetomidine and 1 mg/kg bwt xylazine (YAMASHITA et al. 2000). As the NGD was more reduced with the bolus of xylazine also the following CRI of xylazine (1 mg/kg bwt/h) was more sedative than CRI of dexmedetomidine (5 µg/kg bwt/h), reducing the NGD by 73% and 43%, respectively. In group D2, we reached a comparable NGD to that of xylazine was reached and the resulting CRI of 7 µg/kg bwt/h dexmedetomidine IV reduced NGD by 70%. BETTSCHART-WOLFENSBERGER et al. (1999) reported that a CRI of medetomidine (3.5 µg/kg bwt/h) after a bolus of 5 µg/kg bwt IV induced a constant head drop of at least 50% for two hours. In respect to the fact that in our study 1 mg/kg bwt/h of xylazine induced a greater head drop (74%), it is not
surprising that 3.5 µg/kg bwt/h of dexmedetomidine was not equisedative to it in our sedation trial.

In addition to NGD, we compared reaction to thermal stimulation. Noxious thermal stimulation is considered to be an objective and repeatable method for assessing cutaneous analgesia in horses (KAMERLING et al. 1985; KAMERLING et al. 1988; ROBERTSON et al. 2005). Noxious thermal stimulation of the skin preferentially activates small myelinated Aδ fibres which are responsible for the initiation of pain (“first pain”) and additionally slowly conducting C-fibres, which have been associated with persistent clinical or chronic pain (GAYNOR and MUIR 2009). Results from the present study showed no significant difference between groups, but mean values for thermal threshold after low dose dexmedetomidine were consistently lower compared to the two other treatments. Regarding the absolute values for TTs one has to consider, that the reaction to the thermal stimulation at the area over the right nostril involves movement of the head or at least the ears which might be influenced by the muscle relaxant effect of α2-adrenoceptor agonists. Further a high density of α2-adrenoceptor agonists have been demonstrated in lamina II of the dorsal horn of the spinal cord (BOUCHENAFA and LIVINGSTON 1987), which is an area widely implicated in the transmission of painful stimuli. Noxious stimulation at the area over the right nostril is transmitted via the sensory infraorbital nerve as part of the trigeminal nerve and does not involve the dorsal horn of the spinal cord. Thus, analgesia must basically result from activation of alpha2-adrenoceptors located in the locus coeruleus and the periaqueductal grey mater (GUO et al. 1996; PENG et al. 1996; BUDAI et al. 1998). Application of thermal stimuli at different regions of the body might produce different values for TTs. However, results of thermal stimulation with the same test system as used in the current study at withers (=spinal) and nostrils are not different, but different drugs were used (Diss POLLER 2011, study in preparation).

Based on the results of the sedation trials and information from the literature we decided to test a CRI of 1 mg/kg bwt/h of xylazine and both dexmedetomidine doses
with 5 µg/kg bwt/h and 7 µg/kg bwt/h in combination with a starting dose of 3 mg/kg bwt/h of ketamine and 0.1 mg/kg bwt/h of midazolam for 120 min TIVA.

Our data suggest that cardiovascular function is maintained within acceptable limits for horses anaesthetized with all three protocols. The determination of CO is used as a global indicator of tissue perfusion and to assess the effect of drugs on the circulation. Studies investigating cardiovascular function of various combinations of α2-adrenoceptor agonists (xylazine, detomidine, romifidine, medetomidine), dissociative anaesthetics (ketamine, tiletamine) and central muscle relaxants (guaifenesin, diazepam, climazolam, midazolam, zolazepam) for TIVA in horses demonstrated less cardiovascular depression compared with inhalation anaesthesia (GREENE et al. 1986; BETTSCHART-WOLFENSBERGER et al. 1996; KERR et al. 1996; MCMURPHY et al. 2002; MAMA et al. 2005). In our study CI differed between groups. Since with XKM-TIVA CI increased over the duration of anaesthesia reaching higher values than in the awake horses (130% of baseline), CI was decreased with DKM2-TIVA (78% of baseline). During DKM1-TIVA CI remained fairly constant (between 85 - 101% of baseline). To our knowledge, there are no data available that directly compare influence of α2-adrenoceptor agonists on CI during TIVA. The increase in CI during XKM-TIVA over values obtained in the wake horses is remarkable. CI depends on HR and SV. The decrease in HR was similar in all groups, but SV increased towards the end of anaesthesia with XKM. Stroke volume depends on preload, cardiac contractility, and afterload. Xylazine produces vasoconstriction in the isolated canine pulmonary vein providing evidence that α2-adrenoceptor agonists increase preload (HANIUDA et al. 1989). WAGNER et al. (1991) showed, that preload increases various periods of time after IV administration of xylazine or detomidine and even YAMASHITA et al. (2000) had no evidence for a decrease in preload after medetomidine, detomidine and xylazine administration at different doses. Xylazine and detomidine caused transient reductions in cardiac contractility, which duration was related to the dose and route of administration of the drug (WAGNER et al. 1991). The decrease of SVR with XKM, an indirect indicator of afterload, ran inversely to CI and MAP. It could be a result of a less peripheral arterial vascular constriction that is induced by stimulation of α2-adrenergic receptors in the
vascular smooth muscle after bolus administration of $\alpha_2$-adrenoceptor agonists (BRYANT et al. 1996; LINK et al. 1996; BRYANT et al. 1998). The continuous increase of MAP while SVR decreased supports further that cardiovascular depression was less with xylazine than with dexmedetomidine in our study, although we did not measure cardiac contractility directly. Further investigations are necessary to compare effects of both $\alpha_2$-adrenoceptor agonists on the cardiovascular system.

In our study CO was measured by the lithium dilution technique. A comparison between thermodilution and lithium dilution measurements was performed in anaesthetised foals and mature horses and showed that later method is accurate and reliable (LINTON et al. 2000; CORLEY et al. 2002). The lithium dilution technique seemed to be well suited for the use in equine clinical practice as it does not require a pulmonary artery catheter (CORLEY et al. 2003).

After bolus administration of $\alpha_2$-adrenoceptor agonists, initial hypertension followed by a prolonged hypotension together with a reduced CI and an increased SVR are common (KERR et al. 1972; WAGNER et al. 1991; BETTSCHART-WOLFENSBERGER et al. 1999a; YAMASHITA et al. 2000; KÄSTNER et al. 2001; MURRELL and HELLEBREKERS 2005; KÄSTNER 2006). Dexmedetomidine is not a pure $\alpha_2$-adrenoceptor agonist but also stimulates imidazoline receptors (xylazine does not) that mediate a central hypotension and anti-arrhythmogenic action (HIEBLE and RUFFOLO 1995; FABER et al. 1998) and may be responsible for the lower MAP with DKM1. The higher MAP and SVR with DKM2 suggest, that at the higher dose the peripheral vasoconstrictor action of dexmedetomidine predominates the central hypotensive effect (EBERT et al. 2000).

Respiratory effects were similar between the three TIVA protocols. We decided to give supplemental oxygen with an inspired oxygen fraction of 50 %, because during TIVA with the horse’s breathing room air hypoxaemia can occur (KERR et al. 1996; YAMASHITA et al. 2007). In this study, no horse became hypoxaemic, but in three horses inspired oxygen fraction was elevated up to 75% to maintain arterial $O_2$ tension above 70 mmHg.
At the beginning, the level of anaesthesia did not represent a surgical plane of anaesthesia because almost all horses reacted to nociceptive electrical stimulation. Therefore, the starting dose of 3 mg/kg bwt/h of ketamine would at most allow diagnostic procedures. A ketamine infusion rate between 4 and 4.5 mg/kg bwt/h was sufficient to suppress purposeful movement in response to electrical stimulation in all three groups. To compare clinical effects with pharmacological data, we analysed plasma concentration of xylazine, dexmedetomidine, midazolam and ketamine in group XKM and DKM2. At the beginning of anaesthesia, individual plasma concentrations of ketamine were beyond 1 µg/ml. In literature, at least a plasma concentration of 1.01 ± 0.41 µg/ml of ketamine was necessary for anaesthesia (KAKA et al. 1979). But not only is the concentration of ketamine responsible for surgical anaesthesia. MAMA et al. (2005) evaluated the use of xylazine and ketamine in different dosages and showed that ketamine alone (9.0 mg/kg bwt/h) was not sufficient to prevent response to noxious stimulation, but lower doses of ketamine in combination with xylazine were. In our study, horses did no longer react to electrical stimulation at least at the end of anaesthesia although ketamine concentrations sometimes stayed below 1 µg/ml plasma and plasma concentrations of ketamine are relatively low compared with other studies. The correlation between infusion rate and plasma concentration of ketamine was also low. Neither reaction to noxious stimulation nor the necessity to apply thiopental was linked to special plasma concentrations of ketamine. Interindividual differences of plasma-concentration-time curves of midazolam were also high, despite the clinical homogenous and satisfactorily muscle relaxation during anaesthesia in all horses. It appears, that anaesthesia in horses with a combination of anaesthetics is more complex and the sufficient depth of anaesthesia is not directly linked to one of the plasma concentrations. A reason for the high intra- and interindividual variability might be the inhomogeneity of horses included into the study with an age range of 5 to 23 years and a weight range from 480 to 600 kg.

The quality of recovery ranged from excellent to good in our study with no differences between groups. Previously described protocols for TIVA using midazolam or other benzodiazepine agonists as muscle relaxant resulted in various degrees of ataxia.
after standing (MUIR et al. 2000; GANGL et al. 2001; YAMASHITA et al. 2007). The duration of action of all benzodiazepines is strongly dependent on the duration of their administration (OLKKOLA and AHONEN 2008). The relatively long elimination half-times of all benzodiazepines, a disadvantage for use in TIVA, might be counteracted by the use of specifically acting benzodiazepine antagonists. The elimination half-times and potencies of both, agonists and antagonists, are important to avoid ataxic recoveries and resedation. In our study the elimination half time of midazolam was $t_{1/2} = 50 \pm 22$ min with XKM and $48 \pm 15$ min with DKM2 and hence much lower than reported for humans [102 to 210 min] (ALLONEN et al. 1981; HEIZMANN et al. 1983; GREENBLATT et al. 1984; MISAKA et al. 2010). The onset of effect immediately follows the diffusion of the substance into the CNS and can be observed within the first minutes following flumazenil administration (AMREIN and HETZEL 1990). In humans, the elimination half-life of flumazenil ranged from 40 to 80 min (OLKKOLA and AHONEN 2008). Despite this discrepancy in elimination characteristics between midazolam and flumazenil in humans, flumazenil was sufficient to attain and maintain for about two hours the desired level of consciousness after general anaesthesia (BROGDEN and GOA 1988).

Recovery time was significantly longer with the higher dexmedetomidine dose (DKM2) compared to the lower dose (DKM1). However, the recovery quality was not better after the higher dose which indicates that the longer recovery does not enhance coordination and strength of the horses during the recovery period. Interestingly the same horse within each group was responsible for the upper outliner in recovery duration and quality, confirming that individual character and behaviour of a horse influence recovery quality.

In the literature elimination half-lives of dexmedetomidine in horses are shorter than that of xylazine. For dexmedetomidine, elimination half-life was $19.8 \pm 9.63$ min in mature and $28.96 \pm 7.61$ min in geriatric horses sedated with a bolus of $3.5 \mu g/kg$ IV (BETTSCHART-WOLFENSBERGER et al. 2005). For xylazine, elimination half-life was described as $49.51$ min after bolus administration of $0.6 \text{mg/kg bwt}$ IV (GARCIA-VILLAR et al. 1981). When an anaesthetic drug is administered as an intravenous...
bolus, there is a rapid increase in plasma concentration, followed by rapid distribution and redistribution of the drug throughout the body (MCMURPHY 2005). So it is not remarkable, that \( t_{1/2} \) is longer after infusion of the drug in our study. Elimination half-time of dexmedetomidine was more prolonged than that of xylazine in our study and the assumed advantage of the short acting dexmedetomidine was clinically not visible. The longer elimination half-time together with the longer recovery period is possibly a sign of cumulative drug effects or overdose. In addition, CI was better with XKM than with DKM2 which might have influenced the pharmacokinetics (DUTTA et al. 2000).

6.3 Conclusion and outlook

According to the preliminary sedation trial 1 mg/kg bwt/h after a bolus of 0.5 mg/kg bwt IV xylazine was equsedative to 7 µg/kg bwt/h after a bolus of 3.5 µg/kg bwt IV of dexmedetomidine and had equal analgetic qualities with thermal stimulation. The lower dexmedetomidine CRI of 3 µg/kg bwt/h produced less reduction in NGD and less analgesia.

Cardiopulmonary function was excellent with all three protocols with XKM performing best. However, anaesthetic depth was insufficient at the beginning of anaesthesia in all three groups and 3 mg/kg bwt/h of ketamine was not enough to prevent movement after electrical stimulation. Recovery quality was good to excellent with all three protocols.

During TIVA none of the drugs reached a steady state. Especially with ketamine and midazolam, plasma concentrations showed high inter- and intraindividual variations.

Overall, all three protocols were suitable for two hours of TIVA. An overt advantage of dexmedetomidine over xylazine could not be shown.

In future studies the TIVA protocols should be evaluated under surgical conditions with an increased ketamine infusion rate of 4-5 mg/kg/h, IV.
Summary

Christina Maria Müller

Effects of dexmedetomidine and xylazine on cardiovascular and pulmonary function, recovery quality and duration and pharmacokinetics during total intravenous anaesthesia in horses

The aim of the study was to evaluate the influence of the two α₂-adrenoceptor agonists dexmedetomidine and xylazine on cardiopulmonary function, recovery quality and duration and pharmacokinetics during total intravenous anaesthesia.

Eight adult experimental horses weighing 525 ± 54 kg bwt and aged 13.5 ± 6.8 years were included in this study. The study was carried out as a prospective, randomized experimental cross-over trial. Each horse was sedated (part one of the study) and anaesthetized (second part of the study) three times with at least four weeks between sedation or rather anaesthetic episodes.

During pretrial, equisedative doses of xylazine and dexmedetomidine for constant rate infusion were determined by nose to ground distance and thermal stimulation. Anaesthesia was maintained for two hours. After acepromazine (0.03 µg/kg bwt, IM), xylazine (0.5 mg/kg bwt, IV) or dexmedetomidine (3.5 µg/kg bwt, IV) anaesthesia was induced with ketamine (2.5 mg/kg bwt, IV) and midazolam (0.05 mg/kg bwt, IV). The TIVA was maintained with xylazine (1 mg/kg bwt/h) [XKM] or dexmedetomidine (5 µg/kg bwt/h) [DKM1] or dexmedetomidine (7 µg/kg bwt/h) [DKM2], midazolam (0.1 mg/kg bwt/h) and ketamine (3 mg/kg bwt/h). Ketamine was increased in response to electrical nociceptive stimulation performed every 30 minutes (GRASS-S48-Stimulator). Arterial blood gases, HR, MAP and CI [LiDCOplus-monitor] were measured before treatment (baseline), after sedation, and during anaesthesia. Twenty minutes after the end of TIVA, flumazenil (0.01 mg/kg bwt, IV) was administered. Recovery quality and duration were assessed. Blood samples for kinetic analyses were collected from jugular vein in all groups before and two minutes after sedation as well as 5, 20, 50, 85, 120, 135, 150, 165, 180, 210, 240, 270, 300 and 1440 minutes after CRI was started to determine concentrations of
dexmedetomidine, xylazine, ketamine and midazolam. Plasma drug concentrations were determined by high-performance liquid chromatography. The statistical analysis was performed by two-way analysis with repeated measurements (p < 0.05).

Dexmedetomidine (3.5 µg/kg bwt, IV [bolus] and 7 µg/kg bwt/h [CRI]) was equisedative to xylazine (0.5 mg/kg bwt, IV [bolus] and 1 mg/kg bwt/h [CRI]) concerning nose to ground distance and thermal stimulation. During TIVA MAP was not significantly different from baseline and HR decreased significantly form baseline in all three groups. With XKM CI was not different from baseline at all time points, but had a trend to increase continuously reaching higher values than baseline (88.3 ± 39.2 ml/kg bwt) at 120 min (114.9 ± 30.0 ml/kg bwt) [p=0.68]. Cardiac index decreased significantly after sedation in all groups and remained low with DKM2 during the first hour of TIVA (61.0 ± 17.7 ml/kg bwt and 64.4 ± 15.6 ml/kg bwt). During TIVA CI was significantly higher with XKM than with DKM2. Mean doses of 3.7 mg/kg bwt/h (XKM and DKM2) and 3.6 mg/kg bwt/h ketamine (DKM1) were required. Recovery quality was good to excellent with a mean duration of 37.1 ± 16.1, 31.1 ± 8.9 and 45.5 ± 20.9 min with XKM, DKM1 and DKM2, respectively, resulting in a significant difference between DKM1 and DKM2.

Peak plasma concentrations (C_max) were 1.92 ± 1.41 µg/ml (XKM) and 1.87 ± 1.41 µg/ml (DKM2) for ketamine, 1.28 ± 0.59 µg/ml for xylazine, 4.5 ± 2.45 ng/ml for dexmedetomidine and 0.32 ± 0.21 µg/ml (XKM) and 0.58 ± 0.32 µg/ml (DKM2) for midazolam, respectively. The corresponding values for elimination half-time (t_1/2) were 37 ± 12 (XKM) and 23 ± 2 min (DKM2) for ketamine, 64 ± 13 min for xylazine, 46 ± 7 min for dexmedetomidine and 45 ± 11 (XKM) and 32 ± 3 min (DKM2) for midazolam, respectively. The plasma concentration of ketamine and midazolam showed greater intra- and inter-individual variability than xylazine and dexmedetomidine. None of the drugs reached a steady state. The correlation between infusion rate of ketamine and its plasma concentration during anaesthesia was low (r = 0.37).

In conclusion, all three drug combinations can be used to maintain anaesthesia for two hours with good to excellent recovery. Horses in group DKM2 are more
cardiopulmonary depressed. The characteristics of elimination support the clinically observed shorter duration of recovery with XKM. Therefore, the use of dexmedetomidine has no advantage over the use of xylazine. To achieve an adequate anaesthetic depth, the starting dose of ketamine should be elevated to 4 mg/kg bwt/h. Further investigations under surgical conditions are necessary.
8 Zusammenfassung

Christina Maria Müller

Einfluss von Dexmedetomidin und Xylazin auf die Herz-Kreislauf- und Lungenfunktion, die Aufstehqualität und -dauer, sowie Pharmakokinetik während einer Injektionsanästhesie beim Pferd

Ziel dieser Arbeit war es, den Einfluss der beiden $\alpha_2$-Adrenoceptor Agonisten Dexmedetomidin und Xylazin auf die Herz- und Lungenfunktion, Aufstehqualität und -dauer sowie die Pharmakokinetik während einer Injektionsanästhesie zu untersuchen.

Die Untersuchungen wurden an acht Versuchspferden mit einem Körpergewicht von $525 \pm 54$ kg KGW und einem Alter von $13,5 \pm 6,8$ Jahren durchgeführt. Die Untersuchungen erfolgten nach einer prospektiven, randomisierten experimentellen “cross-over” Verteilung. Jedes Pferd wurde mit einem Abstand von mindestens vier Wochen sediert (Vorversuch) und anästhesiert (Hauptversuch).

Während des Vorversuches wurde die äquipotente Dosierung von Xylazin und Dexmedetomidin für eine Dauertropfinfusion mittels des Abstandes der Nase zum Boden sowie mittels Thermostimulation bestimmt. Nach einer Narkoseprämedikation mit Acepromazin ($0,03 \mu g/kg$ KGW, IM), Xylazine ($0,5 \mu g/kg$ KGW, IV) oder Dexmedetomidin ($3,5 \mu g/kg$ KGW, IV) erfolgte die Narkoseeinleitung mit Ketamin ($2,5 \mu g/kg$ KGW, IV) und Midazolam ($0,05 \mu g/kg$ KGW, IV). Die Narkoseerhaltung erfolgte durch eine konstante Applikation aus Xylazin ($1 \mu g/kg$ KGW/h) [XKM] oder Dexmedetomidin ($5 \mu g/kg$ KGW/h [DKM1] or $7 \mu g/kg$ KGW/h[DKM2]), Midazolam ($0,1 \mu g/kg$ KGW/h) und Ketamin ($3 \mu g/kg$ KGW/h). Die Anästhesie wurde über zwei Stunden aufrechterhalten. Die Ketamin-Dosis wurde bei positiver Reaktion auf eine elektrische nozizeptive Stimulation, die alle 30 Minuten erfolgte, erhöht (GRASS-S48-Stimulator). Mittlerer arterieller Blutdruck, arterielle Blutgase, Herz-, Atemfrequenz und Herzauswurf [LiDCOplus-Monitor] wurden vor der Behandlung, nach Sedation und während der Narkose bestimmt. Zwanzig Minuten nach Ende der Injektionsnarkose wurde Flumazenil ($0,01 \mu g/kg$ KGW, IV) verabreicht. Die
Zusammenfassung


Dexmedetomidin (3,5 µg/kg KGW, IV [Bolus] und 7 µg/kg KGW/h [CRI]) und Xylazin (0,5 mg/kg KGW, IV [bolus] und 1 mg/kg KGW/h [CRI]) waren bezüglich des Abstandes der Nase zum Boden und der Reaktion auf Thermostimulation äquisedativ. Während der Injektionsanästhesie gab es keinen signifikanten Unterschied des mittleren arteriellen Blutdruckes zum Basiswert und die Herzfrequenz sank in allen Gruppen signifikant zum Basiswert. Mit XKM unterschied sich der Herzindex zu keinem Zeitpunkt vom Basiswert, wobei sich eine Tendenz des kontinuierlichen Anstieges zeigte, mit signifikant höheren Werten am Ende der Anästhesie (114,9 ± 30,0 ml/kg KGW) im Vergleich zum Sedationswert (88,3 ± 39,2 ml/kg KGW). In allen Gruppen sank der Herzindex signifikant nach der Sedation, wobei er in der Gruppe DKM2 während der ersten Anästhesiestunde erniedrigt blieb (61,0 ± 17,7 mg/kg KGW und 64,4 ± 15,6 mg/kg KGW). Insgesamt war der Herzindex während der gesamten Anästhesie in der Gruppe XKM signifikant höher als in der Gruppe DKM2. Eine mittlere Dosis von 3,7 mg/kg KGW/h (XKM und DKM2) und 3,6 mg/kg KGW/h (DKM1) Ketamin wurde benötigt. Die Aufstehqualität war in der DKM2-Gruppe mit 45,5 ± 20,9 Minuten signifikant länger als in der DKM1-Gruppe (31,1 ± 8,9 Minuten). Kein signifikanter Unterschied ergab sich zur XKM-Gruppe (37,1 ± 16,1 Minuten).

Aus der pharmakologischen Untersuchung ergaben sich maximale Plasmakonzentrationen von 1,92 ± 1,41 µg/ml (XKM) und 1,87 ± 1,41 µg/ml (DKM2) für Ketamin, 1,28 ± 0,59 µg/ml für Xylazin, 4,5 ± 2,45 ng/ml für Dexmedetomidin und 0,32 ± 0,21 µg/ml (XKM) und 0,58 ± 0,32 µg/ml (DKM2) für Midazolam.
Zusammenfassung


Figure 11: Trial set-up to elaborate the nose to ground distance of a sedated horse.
Figure 12: Plasma concentration [µg/ml] of xylazine in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h), ketamine (mean of 3.7 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 13: Plasma concentration [ng/ml] of dexmedetomidine in horses anaesthetized with a CRI of dexmedetomidine (7 µg/kg bwt/h), ketamine (mean of 3.7 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 14: Plasma concentration [µg/ml] of ketamine in horses anaesthetized with a CRI of xylazine (1 m/kg bwt/h), ketamine (mean of 3.7 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 15: Plasma concentration [µg/ml] of ketamine in horses anaesthetized with a CRI of dexmedetomidine (5 µg/kg bwt/h), ketamine (mean of 3.6 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 16: Plasma concentration [µg/ml] of ketamine in horses anaesthetized with a CRI of dexmedetomidine (7 µg/kg bwt/h), ketamine (mean of 3.7 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 17: Plasma concentration [µg/ml] of midazolam in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h), ketamine (mean of 3.7 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 18: Plasma concentration [µg/ml] of midazolam in horses anaesthetized with a CRI of dexmedetomidine (5 µg/kg bwt/h), ketamine (mean of 3.6 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 19: Plasma concentration [µg/ml] of midazolam in horses anaesthetized with a CRI of dexmedetomidine (7 µg/kg bwt/h), ketamine (mean of 3.7 mg/kg bwt/h) and midazolam (0.1 mg/kg bwt/h).
Figure 20: Plasma concentration of ketamine [µg/ml plasma] in horses anaesthetized with a CRI of xylazine (1 mg/kg bwt/h) [XKM], ketamine and midazolam (0.1 mg/kg bwt/h).
Table 10: Recovery quality scale published by CLARK-PRICE (2008)

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