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The haemodynamic and pharmacologic interaction of medetomidine and peripheral antagonist MK-467 and their dose-dependency in dogs

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ACADEMIC DISSERTATION

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To my family and friends, and
Especially the colleagues whose support was essential
For helping me to find courage to finalise this project

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Abstract

The alpha-2 agonist, medetomidine (MED), and its pure active enantiomer dexmedetomidine (DMED), are used clinically in small animal practice as potent sedatives, analgesic agents, muscle relaxants, and as adjunct agents for balanced anaesthesia. However, their cardiovascular effects limit their use.

The use of constant rate infusion (CRI) for administration of MED was studied in order to provide the sedation and analgesia while decreasing the adverse cardiovascular effects. The second avenue of investigation performed was addition of the peripheral alpha-2 antagonist, MK-467, to limit the haemodynamic effects of MED.

The cardiovascular effects of MED CRI were investigated in a dose-finding study. Six dose levels were administered during general anaesthesia from very-low dose to a high, positive-control dose, in order to quantify dose-dependency. In order to elucidate an appropriate agonist-antagonist dose ratio, a step-down infusion protocol of MED and addition of step-up infusion protocol of MK-467 was performed in anaesthetised dogs. The effects and interaction of both drugs during the absorption and distribution phase were studied during an intramuscular study protocol using a co-administration of both drugs, where three doses of MK-467 were investigated. Plasma concentration measurements provided pharmacokinetic parameter estimates.

MED-CRI administration showed promising results in demonstrating the dose-dependency of the cardiovascular effects. With the low doses of MED CRI, the adverse effects may be minimised, although not completely avoided. A complex pharmacokinetic and pharmacodynamic interaction between the two molecules was revealed; after intramuscular (IM) co-administration of both drugs the absorption of MED was accelerated by the addition of MK-467 to the treatment. The step infusions revealed that MK-467 also increased the elimination of MED. The optimal dose ratio finding is complicated as the context in which the drugs are given (IM, IV, CRI, under general anaesthesia etc.) affects the disposition of MED. The combined pharmacokinetic and dynamic results provide good initial pharmacokinetic estimates for future PK-PD modelling to predict the interaction of these two drugs, MED and MK-467, in dogs.

List of Original publications

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I

Kaartinen J, Pang D, Moreau M, Vainio O, Beaudry F, Del Castillo J, Lamont L, Cuvelliez S, and Troncy E.
Hemodynamic effects of an intravenous infusion of medetomidine at six different dose regimens in isoflurane-anesthetized dogs.
Veterinary Therapeutics 2010, 11(1), pp E1-E16.

II

Kaartinen J, del Castillo JRE, Salla K, Troncy E, Raekallio MR, and Vainio OM.
Haemodynamic interactions of medetomidine and the peripheral alpha-2 antagonist MK-467 during step infusions in isoflurane-anaesthetized dogs.
The Veterinary Journal, 2014, 202(2), pp 353-360.

III

Restitutti F, Kaartinen J, Raekallio MR, Wejberg O, Mikkola E, del Castillo JRE, Scheinin M, and Vainio OM.
Plasma concentrations and cardiovascular effects of intramuscular medetomidine with three doses of the peripheral alpha-2 antagonist MK-467 in dogs.
Veterinary Anaesthesia and Analgesia, 2016, in print.

Abbreviations

A	Adrenaline
AIC	Akaike Information Criterion
BBB	Blood-Brain-Barrier
BP	Blood Pressure
CBF	Cerebral Blood Flow
CI	Cardiac Index
Cl	Clearance
Cl/F	Clearance/bioavailability
Cl_t	Systemic (terminal) Clearance
$CMRO_2$	Cerebral Metabolic Rate of Oxygen
C_{max}	Maximal concentration
CNS	Central Nervous System
CO	Cardiac Output
CRI	Constant Rate Infusion
DAP	Diastolic Arterial Pressure
DMED	Dexmedetomidine
ED_{50}	Effective dose in 50% of the cases
$EtCO_2$	End-tidal Carbon Dioxide
F	Bioavailability
FOCE ELS	First-Order Conditional Expectation with Interaction
HR	Heart Rate
IM	Intramuscular
IV	Intravenous
K_a	Absorption Constant
LMED	Levomedetomidine
LC	Locus Ceruleus
MAC	Minimal Alveolar Concentration
MAP	Mean Arterial Pressure
MED	Medetomidine
MLEM	Maximum-likelihood Expectation Maximisation
NA	Noradrenaline
NCA	Non-compartmental analysis
NPD	naïve pool data
PK	Pharmacokinetic
PD	Pharmacodynamic
PONV	Post-operative nausea and vomiting
QRPEM	Quasi-Random Parameter Expectation Maximisation
SAP	Systolic Arterial Pressure
SVR	Systemic Vascular Resistance
$T_{1/2}$	Elimination half-life
T_{max}	Time to maximal concentration
V_{ss}	Volume of distribution in steady state
V_z	Volume of distribution
V_z/F	Volume of distribution/bioavailability

1. Introduction

The alpha-2 agonist, medetomidine (MED), and its pure active enantiomer dexmedetomidine (DMED), have been used in small animal clinical practice for decades to provide sedation, analgesia, muscle relaxation, and as a part of balanced anaesthesia and analgesia protocols. A multitude of studies, especially in dogs, have looked at their effects on the cardiovascular system, as these adversely affect patient safety, particularly in patients with pre-existing cardiovascular diseases.

Two major avenues of investigation are employed here in order to maintain the desired sedative and analgesic effects of alpha-2 agonists whilst minimising the adverse cardiovascular effects, such as decrease in cardiac output and increase in systemic vascular resistance. First, administration via constant rate infusion (CRI) is used in order to decrease the fluctuation in plasma concentration and provide minimal and stable haemodynamic variation.

Second, the addition of the peripheral alpha-2 antagonist MK-467 is applied to the protocol in combination with the agonist. The effects of the MK-467 molecule are presumed to be on the peripheral alpha-2 receptors as the passage through the blood-brain-barrier (BBB) is very limited. The use of MK-467 has proved a fruitful avenue for many preclinical studies in dogs and also other species, and is proposed to effectively reverse or diminish the agonist effects on the cardiovascular system while only minimally influencing the desired centrally-mediated analgesic and sedative effects.

The research hypotheses were that the effects of MED and MK-467 are dose-dependent; the effects would thus be related to the given dose of each drug and to the achieved plasma concentrations, specifically to the agonist-antagonist plasma concentration ratios. The following literature review will briefly cover the effects of these alpha-2 agonists MED and DMED, and antagonist MK-467, and more specifically any findings related to the dose-dependency of any of their effects.

2. Review of the literature

2.1. Alpha-2 adrenergic receptors

Alpha-adrenergic receptors are divided into alpha-1 and alpha-2 adrenoceptors. Alpha-2 receptors are further divided into three subtypes alpha-2A, -2B, and -2C; see review by (Kamibayashi & Maze 2000). In general, the alpha-2B receptor subtypes in vascular smooth muscle cells mediate the initial vasoconstriction and short-term hypertensive effect, whereas centrally-located alpha-2A receptors mediate the sedative and sympatholytic response and spinal analgesic effect. Centrally-located alpha-2C receptors mediate the anxiolytic and stress-relieving effect associated with alpha-2 receptor agonist administration (Kamibayashi & Maze 2000).

Alpha-2 receptors are widely distributed throughout tissues and organs where they mediate the effects of endogenous catecholamines: adrenaline (A) and noradrenaline (NA). Receptors are located pre-, post-, and extra-synaptically. All three alpha-2 receptor subtypes are transmembrane G-protein coupled receptors, however the subsequent signalling mechanisms coupled to G-protein activation differ between subtypes. Thus, pre-synaptic alpha-2A receptors located at the pons in the Locus Ceruleus (LC) mediate the decrease of NA release and an inhibitory pathway producing sedation and sympatholysis (Doze et al. 1989; Maze & Fujinaga 2000), while alpha-2B receptors located in the vascular smooth muscle mediate a stimulatory response by producing vasoconstriction and hypertension (Kamibayashi & Maze 2000).

Other physiological functions have been found for alpha-2 receptors located post-synaptically in renal, hepatic, pancreatic, and adipose tissues, and in thrombocytes, vascular smooth muscle, as well as in the eye (Scheinin & MacDonald 1989; Murrell & Hellebrekers 2005). The most important physiological functions will be briefly discussed in the following sections on the pharmacodynamic effects.

2.2. Medetomidine – pharmacology

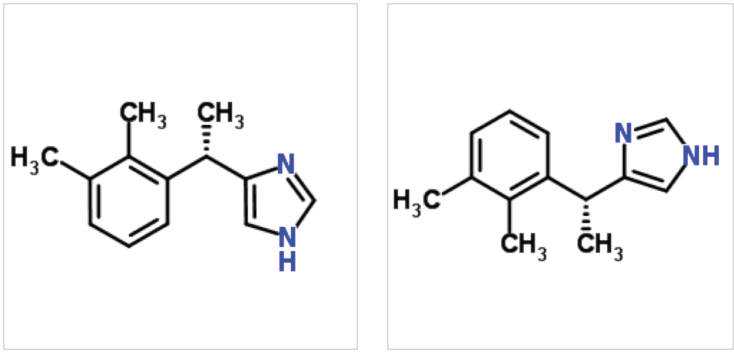

Figure 2.1. Chemical structure of dexmedetomidine and levomedetomidine, (Downloaded from Chemspider.com).

Medetomidine (MED) is a highly potent and selective alpha-2 adrenergic receptor agonist, which has sedative, anxiolytic, muscle relaxant, and analgesic properties (Vainio et al. 1989; Kuusela et al. 2001b; van Oostrom et al. 2011). It reduces requirements for other anaesthetic agents and is widely used for sedation and pre-medication before general anaesthesia in small animals (Murrell & Hellebrekers 2005). The desired sedative and analgesic effects of alpha-2 agonists are mediated via centrally-located alpha-2A receptors (Doze et al. 1989; Maze & Fujinaga 2000), whereas the cardiovascular adverse effects are mediated via alpha-2B receptors situated on the peripheral vasculature (Kamibayashi & Maze 2000).

The molecular name of MED is 4-[1-(2,3-Dimethylphenyl)ethyl]-1H-imidazole (IUPAC) and the chemical structure is presented in Figure 2.1. MED contains one chiral centre and is supplied in a racemic mixture of two optical enantiomers (dexmedetomidine and levomedetomidine), of which dexmedetomidine (DMED) is the active enantiomer (Savola 1989; Virtanen 1989; Kuusela et al. 2000). MED and its active enantiomer DMED have a very high affinity for alpha-2 adrenoceptors where they act as full agonists and possess a selectivity ratio of 1620/1 (alpha-2/alpha-1), which is 5-10 times higher than for xylazine (alpha-2/alpha-1 ratio 160/1) or detomidine (260/1)(Virtanen 1989).

The medetomidine molecule contains an imidazole ring, which also infers affinity to imidazoline-receptors (Kamibayashi & Maze 2000; Murrell & Hellebrekers 2005). The

action mediated via imidazoline receptors is associated with central hypotensive and antiarrhythmic action (Murrell & Hellebrekers 2005) and with protective features against ischemia and reperfusion injury in the CNS (Dahmani et al. 2008; Dahmani et al. 2010; Zhang et al. 2013; Cai et al. 2014).

2.3. Pharmacodynamic effects of medetomidine

2.3.1. General pharmacodynamics

There are a multitude of reports and review papers, only the most essential of which are referred to here, describing the desirable effects of MED as a good quality sedation, analgesia, anxiolysis, and muscle relaxation, anaesthetic sparing effects, and decrease in stress response (Segal et al. 1989; Vainio 1989; Benson et al. 2000; Kuusela et al. 2001b; Väisänen et al. 2002; Sinclair 2003; Murrell & Hellebrekers 2005; van Oostrom et al. 2011). Despite these and other similarly promising reports, MED has remained in relatively restricted use in small animals, and especially in dogs, due to its pronounced cardiovascular effects. The pharmacodynamic effects of MED and DMED action are briefly discussed in the following sections.

2.3.2. Haemodynamic effects

Alpha-2 agonist administration, particularly when administered as rapid bolus IV, has major effects on the cardiovascular system, typically including a biphasic blood pressure response (initial hypertension, followed by normo- or hypotension) with profound bradycardia and decreased cardiac output (CO) and cardiac index (CI), increased systemic vascular resistance (SVR) and central venous pressure (CVP), and bradydysrhythmias (Savola 1989; Vainio 1989; Murrell & Hellebrekers 2005).

After a rapid IV dose or higher doses reaching high plasma concentrations, DMED induces an increase in blood pressure as a result of the activation of peripheral alpha-2 receptors located in the vascular smooth muscle. This increase is temporary as, after redistribution, the peripherally mediated vasoconstriction is overcome by the centrally-mediated reduction of sympathetic activity; see review and original study (Aantaa & Scheinin 1993; Pypendop & Verstegen 1998). With high doses, the increase in

blood pressure will persist for longer (Kuusela et al. 2001a). In dogs, administration of alpha-2 agonists MED or DMED has not been associated with hypotension, although blood pressure may decrease to lower values than before treatment (Pypendop & Verstegen 1998) even if associated with known hypotensive anaesthetic agents such as propofol and isoflurane (Kuusela et al. 2003).

Alpha-2 agonist administration produces a profound decrease in HR and usually a sinus bradycardia is seen with potential bradyarrhythmias, such as first- and second-degree atrioventricular blocks. For example, in one study on dogs, MED given at 40 µg/kg IM decreased HR by 63% (Vainio 1991). Initially, and especially after higher dose administration, the bradycardia is a baroreceptor-mediated response due to increase in blood pressure and increase in afterload (Maze & Tranquilli 1991). Secondly, the decrease in HR is induced by the centrally-mediated sympatholytic effect resulting from reduced NA release in the CNS.

Alpha-2 agonist DMED has demonstrated antiarrhythmic activity, which is at least partly mediated by central alpha-2 receptors (Hayashi et al. 1991). Stimulation of central alpha-2 receptors at the level of LC enhances vagal neural activity, and this in turn prevents sympathetic driven arrhythmias (Kamibayashi et al. 1995a). Further details on the antiarrhythmic effect of DMED, have been provided by blocking the action via imidazoline-receptors and alpha-2 receptors separately (Kamibayashi et al. 1995b). This provides evidence of the involvement of the imidazoline receptor in the antiarrhythmic action of DMED, and furthermore suggests an indirect explanation for the arrhythmogenic action of alpha-2 agonists that lack an imidazole ring in their structure.

A perioperative infusion of DMED in humans during vascular surgery prevents sympathetic activity during emergence from anaesthesia, as demonstrated by attenuation of increases in HR, blood pressure, and plasma NA concentrations (Talke et al. 2000). Other studies including animal models (Roekaerts et al. 1996a; Okada et al. 2007) and human results provide supporting evidence on ‘cardio-protection’, especially in patients who either have, or are at high risk of, developing cardiac disease (Aantaa & Jalonen 2006). A meta-analysis of human trials demonstrated reduced mortality and myocardial infarction with the use of alpha-2 agonists in patients undergoing non-cardiac, vascular surgery (Wijeysundera et al. 2003; Wijeysundera et al. 2009). In addition, the use of

DMED was associated with a trend toward improved cardiac outcomes during non-cardiac surgery, demonstrated by a decrease in mortality and non-fatal myocardial infarction and myocardial ischemia (Biccard et al. 2008). All this evidence suggests that use of infusion of DMED or MED appears beneficial by preventing increases in HR, blood pressure and catecholamine release, thus promoting stable haemodynamic status.

A dramatic decrease in cardiac output (CO) to 25% from the initial values may be seen after alpha-2 agonist administration in dogs (Murrell & Hellebrekers 2005). Several mechanisms accounting for this massive reduction in CO have been suggested. These proposed mechanisms include a direct myocardial depressant effect, alpha-2 agonist induced decrease in metabolic demands, decrease in response to increase in afterload and decrease in HR, myocardial hypoxia and dysfunction in response to coronary vasoconstriction, and decrease in circulating catecholamines in plasma (Housmans 1990; Bloor et al. 1992; Murrell & Hellebrekers 2005). Several of these mechanisms are likely to work together to decrease CO during alpha-2 agonist administration.

In autonomically blocked dogs, the cardiac depressor effect of MED was suggested to be attributable to an increase in peripheral vascular resistance caused by postsynaptic activation of alpha-2 receptors in the peripheral vasculature. In that study, no direct depressant effect on myocardium was produced (Autran de Morais & Muir 1995). Other studies support these findings; DMED in isolated dog hearts (where peripheral alpha-2 receptor mediated response was ruled out) failed to show depressant effects (Flacke et al. 1992). In addition, DMED produces no evident direct negative inotropic or chronotropic myocardial effects (Housmans 1990). Thus the decrease in CO is not a direct depressant effect on cardiac contractility (Autran de Morais & Muir 1995).

The HR decrease seems to be an important factor in the reduction of CO by MED and DMED. Their administration increases SVR triggering a baroreceptor-mediated reflex that decreases the HR. In addition, the sympatholytic response to centrally-mediated alpha-2 effects will decrease the HR. Nevertheless, the decrease in HR may not account for the whole magnitude of CO depression, since anticholinergic pre-treatment (glycopyrrolate), used to correct bradycardia, did not completely abolish the CO reduction (Bloor et al. 1992). It was then suggested that CO decreases as a result of the increased afterload provoked by increased SVR (Bloor et al. 1992). However, this

increase in afterload alone may not account for the degree of CO depression associated with administration of DMED in dogs, as a normal canine heart maintains CO even when afterload is increased in a denervated heart (Zandberg et al. 1984). On the other hand, the CO reduction was prevented when DMED was administered together with a peripheral alpha-2 antagonist, MK-467, which attenuated the increase in SVR, without inhibiting a centrally-mediated decrease in HR (Honkavaara et al. 2011).

Circulating plasma catecholamines are reduced to almost undetectable levels after DMED administration (20 µg/kg IV). This reduction in sympathetic support could also account for some of the reduction of CO (Bloor et al. 1992).

The myocardial perfusion via coronary circulation is controlled to some extent by sympathetic stimulation mediated via alpha-adrenergic receptors (Indolfi et al. 1992). Thus, it has been of interest to study the effects of alpha-2 agonists and antagonists in coronary circulation and myocardial perfusion. However, the results from experimental studies are controversial. DMED appears to play a minimal role in causing myocardial hypoxia, in spite of the fact that it induces vasoconstriction in coronary arteries with associated reduction in coronary blood flow (Coughlan et al. 1992; Indolfi et al. 1992). However, other studies have shown coronary vascular resistance to increase without a change in coronary blood flow (Schmeling et al. 1991). The reduction in coronary blood flow is due to increased coronary vascular resistance and results in increased oxygen extraction from the coronary blood supply (Flacke et al. 1993). However, DMED decreased myocardial energy requirements and oxygen consumption in dogs, which corresponded to the decreased myocardial blood flow and oxygen supply (Lawrence et al. 1996b) and was stated to optimise the blood flow in coronary arteries (Roekaerts et al. 1996b). Thus, in healthy dogs, the oxygen delivery is maintained above the level of oxygen demand of the myocardium. This has been confirmed in healthy human volunteers where the myocardial perfusion decreased in parallel with myocardial oxygen demand, and the systolic performance was also attenuated by centrally-mediated sympatholysis with low-dose DMED CRI and further attenuated by increase in afterload during high dose DMED (Snapir et al. 2006). Furthermore, in an isolated rat heart model, DMED had a ‘cardioprotective’ effect against myocardial ischemia, which was mediated by alpha-2 adrenergic receptors (Okada et al. 2007).

It has been shown that DMED-induced reduction in cardiac output will considerably redistribute the total blood flow to preferentially preserve blood flow to the brain, heart, liver, and kidneys, at the expense of less vital organs (Lawrence et al. 1996a). Furthermore, DMED protected the kidneys against radiocontrast-induced nephropathy by preserving outer-medullary renal blood flow (Billings IV et al. 2008). The combination of MED with midazolam and butorphanol resulted in redistribution of blood flow, measured by laser Doppler flowmetry, as they demonstrated decreased intestinal and skeletal blood flows, while renal cortical blood flow was preserved (Pypendop & Verstegen 2000). Conversely, contrast-enhanced ultrasound showed that there was a decrease in blood flow after DMED in dog kidney, liver, spleen, and small intestine (Restitutti et al. 2013), even though MED (750 mg/m^2) had no effect on glomerular filtration rate (GFR) in conscious dogs (Kushiro-Banker et al. 2013).

DMED decreased the cerebral blood flow (CBF) during halothane anaesthesia in dogs, due to cerebral vasoconstriction without apparent influence on cerebral metabolic rate of oxygen ($CMRO_2$). The decrease in CBF combined with a large decrease in volatile anaesthetic requirements may be useful in neuroanaesthesia when vasodilation and increases in CBF should be avoided (Karlsson et al. 1990). However, the decrease in CBF without a change in $CMRO_2$ may potentially cause cerebral ischaemia, if $CBF/CMRO_2$ ratio is not maintained. Nevertheless, an experiment, using isoflurane in dogs, have confirmed these findings of decreased CBF in the face of little or no change in $CMRO_2$ without showing evidence of global ischemia (Zornow et al. 1990). In contrast, CBF and $CMRO_2$ decreased in parallel and in a dose-related manner with DMED in healthy human subjects, and $CMRO_2$-CBF coupling was preserved during DMED administration (Drummond et al. 2008). Based on these contrasting findings, it may be suggested, that the difference in animal and human studies may be related to species differences in receptor distribution and –population, or that the use of volatile agents halothane and isoflurane even with sub-anaesthetic doses, disrupted the $CMRO_2$-CBF coupling in earlier studies, and/or that the effect is highly dependent on the DMED dose administered. Currently, propofol and sevoflurane are the recommended anaesthetic choices for neuroanaesthesia for maintaining $CMRO_2$-CBF coupling. A low dose DMED may have a role as adjunct to these anaesthetic protocols; DMED decreases the

anaesthetic requirements, prevents haemodynamic alterations of CBF, and provides post-anaesthetic sedation; see review (Farag et al. 2011). In addition, clinical investigations in humans have shown the advantageous effect of DMED on stabilising haemodynamic responses, avoiding emergency agitation, allowing easy extubation without coughing, and providing more comfortable recovery and allowing early neurological examination after intracranial surgeries (Turan et al. 2008).

2.3.3. Analgesic effects

Alpha-2 agonists have been shown to have analgesic action mediated via their receptors (alpha-2A subtype) within the CNS, at the spinal level and possibly at the supraspinal level (Kamibayashi & Maze 2000). These alpha-2 receptors are located diffusely in the nervous system, on the primary afferent nerves, on sympathetic postganglionic neurons, in the dorsal laminae of the spinal cord, and within the brainstem. There are also alpha-2 receptors on smooth muscle and endothelial cells in arteries and microcapillaries. All these are potential sites for analgesic effects in supraspinal, spinal, and at peripheral levels (Poree et al. 1998). To complicate the situation, several receptor subtypes have been implicated in the analgesic effect of alpha-2 agonists. One animal model suggests that DMED hyperpolarizes the membrane potential of substantia gelatinosa (superficial dorsal horn, especially lamina II) neurons by G-protein-mediated activation of potassium channels through postsynaptic alpha-2A and alpha-2C adrenoceptors contributing to antinociceptive effects in the spinal cord (Ishii et al. 2008). Various results have been found in studies depending on the species studied and the methodology used, reviewed by Maze & Fujinaga (2000): alpha-2C receptor subtype has been identified to mediate some nociception in mice in which alpha-2A receptors had been rendered dysfunctional. In rat spinal cord, alpha-2A and alpha-2C receptor subtypes have been identified; alpha-2A subtypes are diffusely distributed while alpha-2C subtypes are located mainly in the dorsal root ganglion, while in humans the alpha-2A and -2B predominate, while alpha-2C were sparse. Finally, PCR studies on human dorsal root ganglion cells showed mRNA of alpha-2B and -2C subtypes, while alpha-2A was relatively deficient (Maze & Fujinaga 2000; Ongioco et al. 2000).

The synergistic effect of alpha-2 agonists and opioids has been studied as both drugs act on the dorsal horn at the spinal cord level. There is additive or synergistic action between these two classes of analgesic drugs, which may depend on the route of administration, IV administration showing additive and spinal route showing synergistic antinociception in rats (Ossipov et al. 1990). Similarly, there was synergism after spinal administration of morphine and clonidine in cats (Omote et al. 1991). In dogs, MED produced significant antinociception that was prolonged and appeared superior with addition of butorphanol compared to either of these drugs administered alone (Grimm et al. 2000).

The alpha-2 agonists and opioids are known to share mechanisms of action: both cause G-protein-coupled inhibition of neurotransmitter release and elicit hyperpolarisation of neurons by altering potassium channel conductance, both receptors are present in the same laminae of the spinal-cord dorsal horn, and both inhibit C-fibre activity. The synergistic and/or additive action may indicate a final common pathway of these drug actions, and synergistic effect measured by decreased spinal reflexes suggests the spinal cord as the site of interaction (Ossipov et al. 1990). There is also some evidence that alpha-2C receptor subtypes may contribute to the nociceptive and opioid synergy effect mediated by alpha-2 receptors in mice (Fairbanks et al. 2002). This synergism was also indirectly shown in a visceral pain model in rats where DMED induced antinociception was attenuated by administration of an opioid antagonist naloxone (Ulger et al. 2009).

The analgesic effect of MED and DMED are dependent on the dose and it is stated that the plasma concentrations required to produce sedation are lower than those required to produce analgesia (Salonen 1992; Kuusela et al. 2000; van Oostrom et al. 2011); the sedative plasma concentrations were approximately 0.5 ng/mL and analgesic was reportedly 2-4 ng/mL. However, when alpha-2 agonists are used as adjunctive analgesic therapy, lower levels may be adequate and also lower plasma concentrations have been shown in humans to alleviate the unpleasantness of pain if not its intensity (Kauppila et al. 1991).

2.3.4. Sedative effects

The sedative effects of MED are mediated via the alpha-2A receptors located in the CNS, more precisely in the LC at the pons and lower brainstem. The LC contains a high number of alpha-2 receptors and here the stimulation of these receptors hyperpolarises the cells and thus neuronal impulse transmission is inhibited (Cullen 1996). Alpha-2 agonists reduce the turnover of NA (MacDonald et al. 1988). The NA is required in the CNS for arousal, and sedation will occur if its release is blocked (Sinclair 2003). The sedative effect was first described via pre-synaptic receptors, but multiple studies have produced evidence of the heterogeneity of central alpha-2 receptors. Thus the distribution and general classification of the alpha-2 receptors in the CNS may not be adequately explained by functional (inhibitory or exhibitory) and anatomical (pre- and post-synaptic) classification (Scheinin & MacDonald 1989; Maze & Tranquilli 1991).

The electroencephalogram (EEG) findings during DMED sedation in humans compared to normal sleep showed similarities to physiological sleep spindles during stage 2 sleep thus activating normal non-REM sleep-promoting pathways (Huupponen et al. 2008). Accordingly, it improves quality of sleep in patients in the ICU by increasing sleep efficiency and stage 2 sleep (Alexopoulou et al. 2014).

Similar to sedative effects, the centrally-mediated anxiolytic effect of DMED has been investigated as this could provide an interesting area of treatment for various situations where stress and anxiety could be prevented or treated without sedation. A rat study on post-traumatic stress disorder showed behavioural and cognitive improvements after DMED treatment compared to placebo (Ji et al. 2014). In dogs, a trans-mucosal DMED gel formulation has recently been approved for treatment of fear and anxiety related to noise aversion (EMA/240374/2015).

2.3.5. Anaesthetic sparing effect

MED and DMED have been shown to reduce the requirements of other anaesthetics including inhalant and injectable drugs. Earlier reports have described a halothane anaesthetic-sparing effect of DMED in rats (Segal et al. 1989) and MED and DMED in dogs (Raiha et al. 1989; Vickery & Maze 1989). This effect was found to be dependent on the dose of DMED given (Vickery & Maze 1989). DMED reduced the MAC of

halothane in dogs by approximately 30%, 60%, and more than 90% with 1, 3, and 10 µg/kg, respectively (Vickery et al. 1988). With higher doses, the anaesthetic sparing is marked and raised the possibility for the selective alpha-2 agonists to act as hypnotics and work as anaesthetics in their own right, which was confirmed in rats and this action was mediated via central alpha-2 receptors (Doze et al. 1989; Maze & Tranquilli 1991). However, this full anaesthetic action is likely not true for dogs. In dogs, MED with a dose of 30 µg/kg IV decreased the isoflurane MAC by 47.2% (Ewing et al. 1993). Two studies on dogs that were given 20 µg/kg DMED IV showed reduced MAC by approximately 90% (Weitz et al. 1991; Bloor et al. 1992). A dose-response quantification of the isoflurane anaesthetic sparing effects with several lower doses of DMED CRIs (0.1, 0.5 and 3 µg/kg/h) in dogs showed dose-dependent decrease in isoflurane MAC by 6%, 18%, and 59%, respectively. (Pascoe et al. 2006). The corresponding arterial plasma concentrations for these decreased isoflurane MAC values were; not measurable, average 0.198, and 1.903 ng/mL, respectively. In human patients, DMED infusions with average plasma concentrations of 0.37 and 0.69 ng/mL, decrease the isoflurane MAC by 35% and 47% (Aantaa et al. 1997).

Anaesthetic sparing effect has also been demonstrated with injectable anaesthetics; there is a dose-dependent reduction of thiopental requirements for induction dose with MED doses of 10, 20, and 40 µg/kg (Young et al. 1990). There is also a dose-dependent reduction of propofol requirements for induction of anaesthesia with MED IM from 5 to 40 µg/kg doses (Hammond & England 1994). More recently, the induction agent alfaxalone requirements were also decreased after DMED administration (3 µg/kg) (Pinelas et al. 2014). Moreover, MED increased the plasma concentration of alfaxalone approximately two-fold compared to when it was administered alone (Bennett et al. 2013)(abstract).

Anaesthetic sparing effect presents some challenges in experimental design when comparing alpha-2 agonist doses during anaesthesia, as maintaining equipotent anaesthetic levels becomes difficult. When various doses of MED or DMED are administered during anaesthesia, the level of anaesthetic sparing for any given dose level should be taken into consideration. When comparing results between alpha-2 agonist

doses, the anaesthetic may influence the pharmacokinetics and pharmacodynamics at different levels if non-equipotent anaesthetic depths are compared.

2.3.6. Respiratory effects

Administration of MED in dogs produced a small depression of respiratory frequency, which is potentially mediated via central alpha-2 receptors (Vainio 1990). Intravenous DMED induced a dose-dependent suppression in the slope of the carbon dioxide response curve, a significant decrease in resting respiratory rate, but without a change in resting $EtCO_2$ in dogs (Sabbe et al. 1994). Although respiratory rate and minute ventilation decreased with MED, arterial blood gas values showed no significant change (Pypendop et al. 1996). In an early study in dogs, MED was shown to elicit no change in blood gas variables and it produced less respiratory depression than isoflurane (Bloor et al. 1989). In spite of similar receptor locations and functions with opioids, MED appears to have no effect on the ventilatory drive by the same mechanism. The effects of narcotics on the ventilatory drive are profound at the dose levels required to produce analgesic effect. In comparison to opioids, MED has analgesic and anaesthetic sparing effects with far less respiratory depression (Bloor et al. 1989). When MED was administered in both halothane and isoflurane anaesthetised dogs, the slope of ventilatory response to carbon dioxide curve increased in dogs anaesthetised with halothane, compared with corresponding values in dogs anaesthetised with isoflurane (Lerche & Muir 2006).

Thus, as MED decreases respiratory rate, minute volume, and respiratory drive in conscious dogs, it should be used cautiously in dogs with CNS depression or pre-existing respiratory problems, and especially when given concurrently with drugs known to depress respiration, such as opioids and isoflurane (Lerche & Muir 2004). When used in combination with opioids and/or benzodiazepines, MED may exacerbate the respiratory depression primarily caused by opioids, and will lead to hypoxia, hypoxaemia (Dart 1999), and compromised tissue oxygenation (Pypendop et al. 1996; Pypendop & Verstegen 1999). Because of these additive or synergistic side effects, oxygen supplementation has been suggested and ventilatory support available when using these combinations (Pypendop & Verstegen 1999).

2.3.7. Gastro-intestinal effects

Administration of MED reduces intestinal motility and gut sounds in small animals (Dart 1999). In fasted dogs, MED (30 µg/kg IV) disrupted the migrating myoelectric complex pattern of the small intestine for approximately 2 hours. It also inhibited colonic motility in fasted dogs, although this inhibition was preceded by a short period of increased muscle tone. In fed dogs, MED (30 µg/kg IV) increased the tone of the proximal colon, while the activity of the medium and distal colon was completely suppressed (Maugeri et al. 1994).

Administration of MED (40 µg/kg IM) inhibited the motility of the gastric antrum, duodenum, mid-jejunum, and ileum. The inhibition of motility was longer in the gastric antrum and the duodenum than in the mid-jejunum and ileum. It also inhibited gastric contractions associated with gastrin secretion (Nakamura et al. 1997). These inhibitory actions on intestinal motility are potentially mediated via peripheral rather than central alpha-2 receptors. In addition, these actions outlast the duration of sedation (Maugeri et al. 1994; Cullen 1996). However, these effects have not been associated to negative clinical outcomes in dogs, and thus may be considered less important alpha-2 actions in this species.

MED has an emetic effect in dogs, particularly after IM administration. Vomiting occurs in 5-20 % of dogs. For example, vomiting occurred in 17 % of dogs receiving any dose studied (Hamlin & Bednarski 1989). With the emesis, there is a potential for aspiration and development of aspiration pneumonia. In addition, vomiting increases intracranial and intraocular pressure, which may be a problem for some patients with cerebral or ocular injury or disease (Lemke 2004). Conversely, in human anaesthesia where post-operative nausea and vomiting (PONV) causes more concern, the avoidance of opioids and using DMED as part of the anaesthetic/analgesic protocol decreased the risk of PONV further than the additional preventive drug therapies (Ziemann-Gimmel et al. 2014).

2.3.8. Other effects

2.3.8.1. Hypothermia

A decrease in body temperature has been associated with alpha-2 agonist administration. The temperature reduction may be centrally mediated, with specific CNS depression, and/or in combination with a non-specific depression of general metabolism and reduction in muscular activity (Virtanen 1989; Verstegen & Petcho 1993). In addition, a direct action on the noradrenergic receptors in the hypothalamus thermoregulatory centre to inhibit thermoregulation may produce thermic alterations in a dose-dependent manner (Cullen 1996). After IV administration, and to a lesser extent, epidural DMED administration, a dose-dependent reduction in core body temperature was observed (Sabbe et al. 1994). However, in other studies MED only slightly reduced rectal temperatures (Pettifer & Dyson 1993; Verstegen & Petcho 1993; Pypendop & Verstegen 1998).

In contrast, the centrally-mediated hypothermic effect may be counterbalanced with peripheral vasoconstriction and central redistribution of blood, with a consequent reduction in cutaneous heat loss compared to the other sedatives and anaesthetic agents that induce vasodilatation (Lemke 2004).

In addition, DMED administration reduced the vasoconstriction threshold and the shivering threshold in healthy human volunteers (Talke et al. 1997b). DMED is thus likely to promote hypothermia but it is also likely to effectively treat shivering. DMED may be used to reduce shivering to prevent increased oxygen consumption during post-anaesthetic rewarming (Doufas et al. 2003).

2.3.8.2. Uterine effects

Alpha-2 agonists were found to increase the contractility of the pregnant and non-gravid uterus in some studies. In a canine study, the effect of MED depended to a higher degree on the level of steroid hormones: A rise in oestrogen levels increased the sensitivity of alpha-2 receptors, while a high level of progesterone during pregnancy stimulated the sensitivity of beta-receptors and decreased the contractility of the uterus. Thus, MED does not appear to promote abortion in pregnant dogs (Jedruch et al. 1989).

2.3.8.3. Effects on glucose balance

Administration of MED (10 or 20 µg/kg) IV in dogs transiently decreased the serum insulin concentration, while plasma glucose concentration remained in the physiological range, although a later small peak of glucose concentrations was evident (Burton et al. 1997). Both MED and xylazine IM increased blood glucose, although MED far less than xylazine (Ambrisko & Hikasa 2002). In addition, premedication with MED (5 µg/kg IM) in dogs with insulinoma, compared to normal dogs, suppressed insulin secretion and increased glucose concentrations in both insulinoma and healthy dogs, thus making a judicious use of low doses of MED an ideal adjunct to anaesthesia during insulinoma removal surgery (Guedes & Rude 2013).

In contrast, DMED (5 µg/kg IV) decreased plasma glucose concentrations substantially 30 min after administration and returned to baseline within 90 minutes (Raekallio et al. 2005). Such a finding had not been described in dogs before. Due to short follow-up, these authors postulated that there could have been a delayed peak in plasma glucose, which was not identified.

Converse, and dose-dependent effects on blood glucose concentration seem more logical, when considering the sympatho-adrenergic system as a whole (Fagerholm et al. 2011). High doses of alpha-2 agonists generally inhibit insulin secretion via pancreatic alpha-2A receptors, whilst with small doses a sympatholytic action of alpha-2 agonists could therefore prevent the adrenaline- or stress-induced increase in glucose concentrations (Sherwin & Sacca 1984) by sympatho-adrenal inhibition of NA and A release (Boyda et al. 2013). In addition, after high doses, the sedation and hypothermia would likely decrease glucose utilisation thus further enhancing hyperglycaemia. Small doses used in humans seem to promote general alpha-2-receptor mediated sympatho-adrenal inhibition (Fagerholm et al. 2011). Other factors contributing to which response the body will produce, include the level of stress and the related sympathetic activation. Thus it is likely that with small doses of DMED or MED administered in stress-free and pain-free dogs the sympatholytic action would predominate the glucose homeostasis associated with decrease or no change in plasma glucose concentrations.

2.3.8.4. Other endocrine effects

Administration of MED increased growth hormone levels in plasma by potentiating its secretion (Hayashi & Maze 1993). However, at clinical doses, this effect is unlikely to have serious consequences (Hayashi & Maze 1993; Sinclair 2003).

A diuretic effect is induced by MED and lasts up to 4 hours (with doses of 10 or 20 µg/kg IV) in dogs (Burton et al. 1998). A diuretic effect is induced in isoflurane-anaesthetised dogs lasting up to 2 hours with MED IV (20 and 40 µg/kg) but not with MED IM (80 µg/kg) (Saleh et al. 2005). A potential mechanism to cause increased production of urine is the MED effect inhibiting the release of vasopressin (= anti-diuretic hormone; ADH) from posterior pituitary and interfering with the vasopressin-mediated water permeability in the renal tubules and collecting ducts (Saleh et al. 2005) although the effects seem dependent on the dose and route of administration. The effect on decreasing vasopressin levels and diuresis was found with MED 20 and 40 µg/kg administered IV but not 80 µg/kg IM. This mechanism has been also reported after administration of other alpha-2 agonists, such as xylazine in rats (Cabral et al. 1997; Menegaz et al. 2000). However, a study in dogs administered DMED 5 µg/kg IV, demonstrated no significant change in vasopressin concentrations during 90 minutes after drug administration (Raekallio et al. 2005). In addition, other mechanisms may also be involved, such as systemic vasoconstriction and increased systemic blood pressures followed by increases in RBF and GFR (Cabral et al. 1998; Pypendop & Verstegen 2000; Saleh et al. 2005), and inhibiting the release of insulin inducing glucosuria and osmotic diuresis (Crighton 1990). However, in canine studies, plasma glucose concentrations failed to exceed the renal tubular maximum for glucose reabsorption (Burton et al. 1997; Saleh et al. 2005) and thus glucosuria may not play a part in alpha-2 agonist induced diuresis. Thus, the diuretic effect of MED and DMED appears to be dose- and route-dependent.

Molecules that contain an imidazoline ring (for example etomidate, MED, and DMED) can inhibit hydroxylase enzymes involved in the production of adrenocorticosteroids (Maze et al. 1991; Tobias 2007). In dogs receiving DMED (80 µg/kg IV), basal cortisol levels decreased and the cortisol response to ACTH was blunted 3 h after DMED administration (Maze et al. 1991). In dogs, administration of MED (20

µg/kg IM) transiently decreased plasma cortisol concentrations (Väisänen et al. 2002) while DMED (5 µg/kg IV) only decreased plasma cortisol concentration after exercise which may indicates different responses depending on the level of stress before drug administration (Raekallio et al. 2005). Low dose DMED in human patients provided no evidence to suggest depressed adrenocortical function (Venn et al. 2001) to the extent that occurs, for example, with etomidate. Thus, this effect is likely of little clinical significance and is most likely dose-dependent in dogs.

DMED decreases interleukin-6 levels from baseline (Nishina et al. 1999). This was confirmed in human ICU patients requiring sedation (Venn et al. 2001). DMED also attenuates the systemic inflammatory response during endotoxaemia (Taniguchi et al. 2004).

2.3.8.5. Ischaemia and reperfusion injury

A recent review brings together all potential and investigated molecular targets and mechanisms of action of DMED in ischemia-reperfusion (I-R) injury (Cai et al. 2014); altogether, DMED modulates gene expression, ion channel activation, transmitter release, inflammatory processes, apoptotic and necrotic cell death, and protects cells against ischemia-reperfusion injury via various mechanisms. DMED has also been investigated during I-R injury in critical organs including intestine, myocardium, kidney, lung, brain, and liver, the most essential findings are mentioned below.

A possible neuroprotective effect of alpha-2 agonists was hypothesized following the improvement of neurologic outcome after cerebral ischemia with the use of alpha-2 agonists (Maier et al. 1993; Iida et al. 2006). The improved haemodynamic stability via sympathetic blockade and/or the decreased release of excitatory neurotransmitters (such as glutamate) produced by alpha-2 agonists may account for the neuroprotective effects during and after ischemia (Dahmani et al. 2005; Tobias 2007).

In anaesthetized dogs, DMED preserved coronary blood flow in post-ischaemic hyperaemic subendocardial layer and also reduced the determinants of myocardial oxygen demand (Roekaerts et al. 1996b). In a rat heart model, DMED decreased coronary blood flow and significantly decreased myocardial infarct size after ischemia and reperfusion (Okada et al. 2007). In pigs, DMED administration before ischaemia-

reperfusion injury reduced the incidence of ventricular arrhythmias, improved the recovery of ventricular contractility indices, and suppressed the NA concentration in plasma after reperfusion (Yoshitomi et al. 2012).

Ventilator-induced lung injury (VILI) was mitigated in dogs when DMED was administered through regulation of inflammatory molecules contributing to the injury (Chen et al. 2014). In rats, DMED was shown to attenuate lung I-R injury and yohimbine (a non-specific alpha-2 antagonist) failed to completely antagonise this protective effect (Jiang et al. 2014). Thus, mechanisms mediated via different pathways than alpha-2 receptors may be responsible for this effect of DMED.

The use of DMED at high doses alone or in small doses as an anaesthetic adjunct improved renal I-R injury in rats (Sugita et al. 2013). DMED protected against sepsis-induced acute kidney injury in mice (Hsing et al. 2012) and in rats (Tan et al. 2015) and the mechanism was proposed to be downregulation of inflammatory reactions mediated via alpha-2 receptors as all the protective effects could be reversed by yohimbine (Tan et al. 2015).

2.4. Dose-dependency

The intensity of the cardiovascular effects of MED following rapid IV bolus administration have been previously reported to not follow a clear dose-response relationship in conscious dogs (Pypendop & Verstegen 1998). The duration of these effects was dose-dependent, however. Failure to reveal such dose-response relationships could be related to the high level of inter-individual variation in the disposition pharmacokinetics of the drug. Nevertheless, the aim of dose-dependent decreases in the undesired hemodynamic effects has popularized the use of MED or DMED low rate IV infusions in an attempt to improve the cardiovascular safety. Several reports in the veterinary literature describe the potential benefits of this administration strategy in dogs (Grimm et al. 2005; Pascoe et al. 2006; Lin et al. 2008; Uilenreef et al. 2008; Valtolina et al. 2009; Pascoe 2015).

Despite the quantification of the minimum alveolar concentration (MAC) sparing effect of DMED CRI (Pascoe et al. 2006), the dose-response relationships of MED or DMED on the degree of cardiovascular depression remained to be adequately

determined, which was a requisite for establishing an optimal dose for CRI during general anaesthesia. Four studies have been published investigating cardiovascular effects of DMED CRIs in dogs under general anaesthesia and one study looked particularly at the analgesic effect. In the first clinical study, 3 doses (1, 2 and 3 μg/kg/h) of DMED resulted in acceptable mean arterial blood pressure (MAP) with no changes in circulating lactate concentrations suggesting adequate tissue perfusion (Uilenreef et al. 2008). A second, experimental study, using a single dose (25 $\mu g/m^2/h$) of DMED CRI compared cardiovascular and respiratory effects between propofol and isoflurane anaesthetic groups (Lin et al. 2008). These studies both reported adequate oxygen delivery and a significant effect of general anaesthetic on HR, vasoconstriction, and CI. A third study compared two CRI doses of DMED, a low dose (0.5 μg/kg/h) and a high dose (3 μg/kg/h) during equipotent isoflurane anaesthesia, and concluded that the low dose would be useful during anaesthesia as cardiovascular effects remained acceptably low while some anaesthetic sparing effect was maintained (Pascoe 2015). The fourth study continued along the same investigation lines and compared a single dose (25 $\mu g/m^2/h$) of DMED CRI to morphine CRI in per- and post-operative ICU patients (Valtolina et al. 2009). These authors concluded that DMED produced equally effective analgesia with morphine without increases in adverse effects.

2.5. Pharmacokinetics of medetomidine and dexmedetomidine

Usually MED administration in dogs is either via the intramuscular (IM) or intravenous (IV) route, as the subcutaneous route is considered less reliable (England & Clarke 1989). In dogs, after IM administration, absorption half-life is rapid, around 7 minutes, and peak serum concentrations are reached within 30 minutes; the apparent volume of distribution after a high dose (80 μg/kg IM) is 3.0 Litres; the elimination half-life is 1.28 hours and clearance 27.5 mL/min/kg (Salonen 1989). The clearance of LMED enantiomer has been reported to be faster than that of DMED or racemic MED when pure enantiomers are administered either together or separately (Kuusela et al. 2000). Protein binding is high, approximately 85-94% is protein-bound and thus the free fraction available for distribution to the tissues is limited. When administered IV, the elimination half-life is faster (0.97 hours), compared to IM (Salonen 1989). As expected from pharmacokinetic

findings, after IV bolus administration, the onset of pharmacodynamics action is fast and the peripheral cardiovascular effects appear more pronounced than by IM administration (Pypendop & Verstegen 1998). MED and DMED are highly lipophilic, thus readily absorbed and rapidly distributed to high perfusion tissues such as the brain (Salonen 1992) although the vasoconstrictive effects may decrease the absorption after IM administration. Pharmacokinetic models that have been used to describe the MED or DMED kinetics in dogs are two-compartmental and non-compartmental models with relatively high inter-individual variability (Kuusela et al. 2000; Honkavaara et al. 2012).

MED and DMED metabolism is via hepatic biotransformation, and the elimination is mostly regulated by the blood flow to the liver (Salonen 1992). MED is mainly (80-90%) metabolized by hepatic hydroxylation followed by glucuronidation in dogs, involving several biotransformation pathways (Salonen 1992). The phase I reaction proceeds with a rate sufficient for the rapid removal of the drugs from the body and is regulated mainly by the hepatic blood flow. The phase II glucuronidation of MED with glucuronic acid is accomplished by different UDP-glucuronosyltransferases with different affinity, regio-and stereo-selectivity in human and canine liver microsomes, leading to N-glucuronidation of LMED and DMED with different kinetics (Kaivosaari et al. 2008). In addition, an O-glucuronidation pathway has been reported for MED (Salonen 1992). A phenotypic polymorphism of the cytochrome P450 (CYP)-catalysed phase I hydroxylation of MED, that affects its biotransformation rate has been reported in rabbits (Avsaroglu et al. 2008). Duhamel et al., (Duhamel et al. 2010) reported that MED in canine hepatic microsomes *in vitro* showed a relatively low enzyme saturation and hepatic clearance that may limit the rate of metabolism. In addition, they suggested that the principal metabolic pathway is via CYP3A isoenzyme, the same enzyme responsible for fentanyl, ketamine, and midazolam metabolism, and of which ketoconazole is a known inhibitor able to prevent MED hydroxylation. All in all, species differences in MED metabolism kinetics certainly exist (Kaivosaari et al. 2002) thus predictions across species should be made carefully.

In a pharmacokinetic model for humans, the decrease in cardiac output (CO) decreased the drug elimination clearance for DMED (Dutta et al. 2000), which supports the postulate that the blood flow to the liver limits elimination. Thus, the cardiovascular

effects of DMED by decreasing CO attenuate its own elimination kinetics (Dutta et al. 2000). In addition, other reports support this finding as it has been shown that reversal of MED with alpha-2 antagonist atipamezole reduced the elimination half-life of MED (Salonen 1992) and DMED clearance was accelerated by the addition of the peripheral alpha-2 antagonist MK-467 (Honkavaara et al. 2012). Inspired by the human model by Dutta et al. (2000). Pypendop et al (2013) modelled the pharmacokinetics of DMED in cats, and a model where clearance was proportionally affected by the decrease in CO was tested and compared to a generic two-compartmental model. This model is simplified as in addition, the product of unbound drug available for elimination and intrinsic clearance would also affect the clearance as it was affected less than predicted by CO decrease alone. However, addition of this parameter worsened the prediction made by the model (Pypendop et al. 2013).

In adult humans with severe hepatic failure, the volume of distribution in steady state and elimination half-life of DMED were increased and clearance was decreased (Cunningham et al. 1999). In human adults with severe renal disease, there was no significant difference between renal disease and control patients for volume of distribution and clearance. However, the elimination half-life was potentially decreased with renal disease, while the perceived clinical sedation was prolonged (De Wolf et al. 2001). In children, the pharmacokinetic profile appears similar to adults except in very young infants, for which conflicting data have been demonstrated. Very young infants have been reported to clear DMED faster (Tobias 2007). However, in another study using population analysis techniques, clearance in neonates was approximately one-third of that described in adults (Potts et al. 2008). Two studies in paediatric patients showed either comparable pharmacokinetic data to adults (Diaz et al. 2007), or slightly different data in respect of larger weight-adjusted volume of distribution in steady state in children younger than 2 years of age (Vilo et al. 2008), resulting in a need for larger loading doses in younger infants.

An earlier study on DMED pharmacokinetics of IM and IV DMED on healthy human adult volunteers, demonstrated that plasma concentration versus time data best fitted a three-compartment model and the patient height also affected the kinetics (Dyck et al. 1993). Another study of IV DMED in women after a single dose was shown to

follow two-compartment kinetics, as the plasma concentration versus time curves failed to support the use of a third compartment and patient demographics did not alter kinetics (Talke et al. 1997a). The latter authors suspected the differences between these two studies could have been in the sampling times that the latter lacked the ability to detect the early distribution phase of the drug and proposed that as women had less height differences they could not show a difference on this aspect. Further study looking into effect of CO on the pharmacokinetics of DMED, found a two-compartmental model best fitted the data in men who were given a DMED CRI with a computer-controlled infusion pump (Dutta et al. 2000). A population pharmacokinetics model was used in ICU patients administered long-term DMED infusions (Iirola et al. 2012). In their model, in addition to CO, the patient age and low albumin concentration affected the PK of DMED and prolonged the elimination half-life and the context-sensitive half-time.

2.6. Peripheral alpha-2 antagonist MK-467 – pharmacology

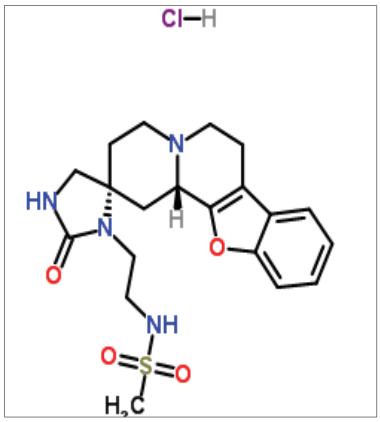

Figure 2.2. Chemical structure of MK-467 (Downloaded from Chemspider.com).

The molecular name of MK-467 is N-[2-[(2R,12bS)-2'-Oxo-1,3,4,6,7,12b-hexahydro-3'H-spiro[1-benzofuro[2,3-a]quinolizine-2,4'-imidazolidin]-3'-yl]ethyl] methanesulfonamide hydrochloride (1:1) (IUPAC) and its chemical structure is presented in Figure 2.2. The alpha-2 antagonist MK-467, also previously named as L-659,066, acts mainly on peripheral alpha-2 receptors because of its minimal capacity for crossing the blood-brain-barrier, as has been initially demonstrated in rats and marmosets

(Clineschmidt et al. 1988). Currently, there is no product of MK-467 available for clinical use.

2.7. Pharmacodynamic effects of MK-467

Peripheral alpha-2 antagonists have been studied in animal models and in human volunteers for their potential therapeutic use for improving insulin release in diabetic patients and enhancing lipolysis without the complicated centrally-mediated effects on sympathetic outflow (Sciberras et al. 1994). In spite of these interests, in dogs it would probably be of little clinical use on its own but it has been studied extensively; mostly in an attempt to attenuate the adverse cardiovascular effects of alpha-2 agonists mediated via receptors on the peripheral vasculature in dogs (Enouri et al. 2008; Honkavaara et al. 2008; Honkavaara et al. 2011; Restitutti et al. 2011; Honkavaara et al. 2012; Rolfe et al. 2012).

2.7.1. Haemodynamic effects

Pagel et al. (1998) raised interest in the peripheral alpha-2 antagonists by reporting attenuation of the early cardiovascular effects of DMED with several doses of intravenous (IV) MK-467 administered prior to DMED in conscious dogs. In another study, a simultaneously administered IV injection of MK-467 attenuated the cardiovascular effects of DMED in dogs (Honkavaara et al. 2011). On the other hand, it has been demonstrated that MK-467 interfered minimally with the centrally-mediated clinical sedative effects of MED or DMED (Honkavaara et al. 2008; Rolfe et al. 2012). Conversely, MK-467 was shown to reduce the effect of DMED on the bispectral index in dogs (Restitutti et al. 2011) however without apparent reduction of the sedation scores.

Thus far, only Rolfe et al. (2012) have reported on the effects of a combination of MED and MK-467 after intramuscular (IM) administration. They demonstrated attenuation of the cardiovascular effects of MED by MK-467 (MED: MK-467 dose ratio of 1:20) when the drugs were injected separately. Furthermore, Salla et al. (2014b) demonstrated attenuation of the cardiovascular effects of the combination MED and butorphanol by MK-467 after both IV and IM routes. Rolfe et al (2012) did not report the plasma concentrations of these drugs after concomitant IM, thus they could not evaluate

whether the effects were consequences of pharmacokinetic or pharmacodynamic interactions.

Although most literature refers to MK-467 as an alpha-2 receptor antagonist, it also produces some actions on its own, as it has been shown to increase heart rate and lower systemic vascular resistance in dogs (Pagel et al. 1998) and to decrease systemic blood pressures in rats (Szemeredi et al. 1989). In addition, MK-467 administered alone increased heart rate, cardiac index, and tissue oxygen delivery in dogs (Honkavaara et al. 2011). In horses, MK-467 alone increased intestinal motility and defecation and in some cases softened the appearance of faeces (de Vries et al. 2016). Thus, it appears that MK-467 is not completely void of pharmacological effects, but rather initiates responses similar to an inverse agonist by binding to the same receptor as an agonist but inducing pharmacological responses opposite to that of exogenous (MED or DMED), or endogenous agonists (NA). Although this may be theoretically sound, the actions could also be categorised as antagonist effects on the adrenergic system via alpha-2 receptors by blocking the endogenous agonists activity on sympathetic tone, thus seemingly eliciting effects of its own.

2.7.2. Effects on alpha-2 receptor mediated analgesia

The analgesic effects mediated via alpha-2 receptors are mostly believed to occur in the CNS, predominantly in the dorsal horn of the spinal cord, having synergistic action with opioid analgesia (Fairbanks et al. 2002). Thus, a peripherally acting antagonist MK-467 should have limited effect on centrally-mediated analgesia and it was shown in rats that it has no effect on DMED-induced visceral analgesia (Ulger et al. 2009). The same report also showed the synergism with opioid receptors as the DMED induced antinociception was attenuated by administration of an opioid receptor antagonist naloxone.

Conversely, MK-467 has been demonstrated to reduce the somatic antinociceptive effect of MED to ineffective levels (Bennett et al. 2016b). This recent report demonstrated a pharmacokinetic interaction causing approximately halving of the DMED plasma concentrations to be available to reach the central receptors when MED (10 µg/kg) and MK-467 (250 µg/kg) were co-administered IV. The MK-467 co-administration decreased DMED plasma concentrations lower than required to produce

analgesia (Bennett et al. 2016b). However, there are no studies on the spinal cord penetration of MK-467 in dogs, or on the potential other alpha-2 receptor sites of analgesic action, thus direct antagonism may still be possible. Nevertheless, based on this investigation, it is suggested that a higher dose of MED should be used to maintain analgesic effect when combined with MK-467.

In addition, it was shown in a neuropathic pain and induced hyperalgesia model in rats, that first of all the analgesic efficacy (and ED_{50}) of DMED had a left shift by three to six fold, while there was no shift for the sedative effect compared to normal rats (Poree et al. 1998). Secondly, a pre-treatment with the peripheral antagonist had no effect on the analgesic effect in normal rats, while it effectively antagonised the analgesic effect of DMED in neuropathic rats. These authors suggested that the nerve ligation induced neuropathy might have caused both sensitisation to alpha-2 receptor-mediated analgesia and induced a novel site of alpha-2 analgesia outside the BBB. Thus, it appears that some types of pain, such as experimentally induced neuropathic pain, may not be controlled with the use of peripheral alpha-2 antagonist in combination with an alpha-2 agonist.

2.7.3. Effects on alpha-2 receptor mediated sedation and anaesthetic sparing effects

Most studies have shown no detectable effects of MK-467 on centrally-mediated effects, such as sedation scores produced by alpha-2 agonists (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012). However, when using an objective modality, such as bispectral Index (BIS), as a measurement of sedation or loss of consciousness, it was affected by addition of MK-467 on DMED administration, although the researchers postulated that this could be explained by the effect on the electromyogram (EMG) that is known to affect BIS although their study did not show differences in EMG during the differences between BIS levels (Restitutti et al. 2011). The potential effect of MK-467 on DMED pharmacokinetics decreasing the amount of DMED available to reach the CNS could be another potential reason (Honkavaara et al. 2012). In fact, this has been shown in a recent report where MK-467 attenuated the sedative effect of MED and also decreased the duration of sedation and these authors demonstrated that the most likely effect would be due to pharmacokinetic interaction rather than actual central penetration of MK-467 (Bennett et al. 2016b).

2.7.4. Other effects

The effects of MED and DMED on glucose balance are at least partly mediated via the peripheral receptors in the pancreas inhibiting insulin secretion. Thus the peripherally acting MK-467 can antagonise these effects, as has been shown in dogs (Restitutti et al. 2012). In addition, a high dose was shown to produce hypoglycaemia in mice (Durcan et al. 1991). A study in healthy human volunteers, however, found no effect of MK-467 administration, alone, on glucose and insulin balance (Schafers et al. 1992).

One report describes the effect of MK-467 on hypothermia to be lacking in mice (Durcan et al. 1991), thus supporting the evidence that this effect is mediated via central alpha-2 receptors that this peripheral antagonist has poor access to. Nevertheless, if MK-467 is given with an alpha-2 agonist, it will prevent the peripheral vasoconstriction and thus enhance heat loss as has been demonstrated in dogs (Vainionpaa et al. 2013a).

2.8. Pharmacokinetics of MK-467 and its effect on pharmacokinetics of MED and DMED

Few pharmacokinetic studies on MK-467 have been published. One recent *in vitro* study reported the protein binding of this molecule in canine plasma to be 72-74% in clinically-relevant dose ranges and the addition of MED did not alter the protein binding of MK-467 (Bennett et al. 2016a). Reported mean pharmacokinetic parameters for conscious dogs after administration of 250 µg/kg MK-467 IV alone were the following in two different studies, respectively: volume of distribution (V_z) 0.41 and 1.0 L/kg; clearance (Cl) 7.8 and 10.6 mL/min/kg; half-life 39 and 65.6 minutes (Honkavaara et al. 2012; Bennett et al. 2016b).

MK-467 was shown to alter the early disposition of DMED following concomitant IV administration, by doubling the apparent clearance and the apparent volume of distribution of DMED (Honkavaara et al. 2012). Similar results have been confirmed for combined administration of MED and MK-467 as addition of MK-467 lowered both enantiomers DMED and LMED plasma concentrations, decreased elimination half-life, and increased volume of distribution, and clearance (Bennett et al. 2016b). In addition, in horses, administration of detomidine or romifidine with MK-467

revealed that MK-467 decreased the maximal plasma concentrations and increased the volume of distribution and clearance of the given alpha-2 agonists (Vainionpaa et al. 2013b; de Vries et al. 2016).

3. Aims of the studies

1) To characterise the dose-dependency of the effects of medetomidine (I, II) and MK-467 (II, III).

2) To detect the plasma concentrations of medetomidine and MK-467 and to link the DMED:MK plasma concentration ratios to the physiological responses (I-III).

3) To document the interaction of medetomidine and MK-467 on the pharmacokinetic, cardiovascular, and sedative effects in order to find the optimal dose ratio (II, III).

4. Materials and Methods

4.1. Animals

In study I, twenty-four healthy purpose-bred laboratory beagles (13 spayed females and 11 neutered males) were used. These dogs were between 1 and 3.5 years old and weighed between 9 and 16 kg. In study II, eight healthy purpose-bred laboratory beagles (6 neutered males and 2 spayed females) were used, and were all aged 4.5 years and weighed between 12 and 16 kg. Another group of eight dogs (6 neutered males and 2 spayed females) was used in study III, and they were all one year of age and weighed between 11 and 15 kg.

For each study, the care and use of the dogs complied with National Council for Animal Care and The National Animal Experimentation Committee approved the study protocols for each study. Prior to the experiments, food was withheld for 12 hours with free access to water. All dogs were accustomed to handling and instrumentation.

All dogs were housed in groups in large pens. Commercial dog food was given once or twice daily and water was freely available. All the dogs were routinely health checked, vaccinated, and dewormed. The health status was based on clinical examination and blood haematology and biochemistry values prior to each study.

4.2. Study designs and treatments

The study protocols performed in each experiment are summarised in Table 4.1.

Table 4.1. The summary of study protocols.

Study	Number of animals	Number of treatments & (n = X)	Treatments	Primary Outcome	Secondary Outcome
I	24	6 n = 4	MED CRI 1) 0.2 µg/kg/h 2) 0.5 µg/kg/h 3) 1.0 µg/kg/h 4) 1.7 µg/kg/h 5) 4.0 µg/kg/h 6) 12 µg/kg/h	Haemodynamic effects under ISO anaesthesia	PK: IV CRI
II	8	2 n = 8 (Crossover design)	1) MED CRI step-down + Placebo 2) MED CRI step-down + MK-467 step up	Haemodynamic effects under ISO anaesthesia; Interaction	PK: IV Infusions; Interaction
III	8	4 n = 8 (Crossover design)	1) MED 20 µg/kg IM 2) MED 20 + MK-467 200 µg/kg IM 3) MED 20 + MK-467 400 4) MED 20 + MK-467 600	Haemodynamic; Interaction	PK: IM and sedation; Interaction

In study I, each dog received one of 6 treatments (6 groups, n=4 per group), in a randomised, prospective, control phase, and 'blinded' design. Medetomidine hydrochloride (Domitor; Orion Pharma, Espoo, Finland) was administered over 10 min at a manual loading dose of 0.2, 0.5, 1.0, 1.7, 4.0, or 12.0 µg/kg, followed by a 60 min maintenance CRI at automated rates of 0.2, 0.5, 1.0, 1.7, 4.0, or 12.0 µg/kg/h, respectively. Instrumentation, baseline measurements, and actual study protocol were all performed under stable isoflurane (ISO) anaesthesia including the 70 min-long treatment of MED combined with ISO, and again a 60 min follow-up (ISO alone) after the end of MED CRI.

In study II, each dog was administered each of two treatments in a randomised, crossover design with a 14-day washout period between treatments. Similar to study I, instrumentation, baseline measurements, and study protocol were all performed under controlled ISO anaesthesia. Medetomidine hydrochloride (Dorbene, Syva Laboratorios, S.A., Spain) was administered with placebo (0.9% saline, Fresenius Kabi) (MED) or with MK-467 (Merck, Sharp & Dohme, PA, US) (MMK). In both treatments, MED was administered over 1 min as a loading dose of 1.25 µg/kg, while simultaneously beginning

a step-down infusion at the following rates: 8.0 µg/kg/h (step 1: 0–20 min), 5.5 µg/kg/h (step 2: 20–40 min), and 4.0 µg/kg/h (step 3: 40–95 min). Five minutes after the initiation of MED administration, MK-467 or placebo was given with a step-up infusion protocol. The protocol was as follows: 100 µg/kg/h (step 1: 5–35 min), 200 µg/kg/h (step 2: 35–65 min), and 500 µg/kg/h (step 3: 65–95 min). Both drug infusions were terminated at the same time (95 min).

In both studies I and II, MED was diluted with 0.9% saline as appropriate, and administered automatically with an infusion pump or syringe driver. In study I, the MED loading doses were prepared in 3-mL syringes by a laboratory assistant who was aware of the treatment and they were administered manually over 10 minutes by the researcher unaware of the treatment protocol. Study II was 'non-blinded', and the loading doses were drawn from the prepared infusion bag, and given manually over 1 minute. In study II the MK-467 powder was diluted with 0.9% saline to a final concentration of 0.1 mg/ml, and administered with a syringe driver similar to the placebo.

Study III differed from the first two studies as the drugs were administered as IM bolus instead of IV CRIs in order to also evaluate the effects during the absorption phase of the drugs. Study III was a randomized, four-period crossover, experimental study, where the main evaluator remained aware ('non-blinded') of the treatment groups. Each dog was administered all four treatments (1. – 4.) with a minimum washout period of 14 days between treatments:

1. Medetomidine 20 µg/kg IM (Dorbene, Syva Laboratories S.A., Spain) (=MED),
2. MED and MK-467 (MK-467 powder, PCAS, Finland) 200 µg/kg IM (MK200),
3. MED and MK-467 400 µg/kg IM (MK400),
4. MED and MK-467 600 µg/kg IM (MK600).

The MED was drawn directly from the commercial preparation of 1 mg/ml concentration. MK-467 powder was diluted with physiological saline solution immediately prior to administration to a concentration of 10 mg/ml. Once both drug doses were prepared they were then mixed in one syringe for a single injection.

4.3. Study procedures

Studies I and II were performed under general anaesthesia while during study III, the dogs were instrumented under anaesthesia and then allowed to wake up for the actual assessment period. During study I, each treatment was initiated by mask induction with ISO in oxygen, and in study II and III one intravenous cannula was placed first and anaesthesia was induced with 4–6 mg/kg propofol IV (Propovet 10 mg/mL, Abbott Laboratories, UK). After induction of anaesthesia the tracheas were intubated and anaesthesia was maintained by constant end-tidal ISO level (ETISO) at 1.3-1.4%, and the $ETCO_2$ was maintained at normal levels between 35-45 mmHg by controlled ventilation (I and II). For each experiment, instrumentation required an arterial cannula to be placed in a peripheral artery, to directly monitor the systemic blood pressures, cardiac output (CO), and/or to collect arterial blood samples. Two venous cannulas were required in each cephalic vein: one for MED infusion (I and II), and the other to allow administration of fluids, placebo, or MK-467 infusions (I-III). A cannula was also placed in a jugular vein for peripheral jugular venous blood sampling in study I and a central venous cannula was placed via the jugular vein instead in studies II and III to also allow for continuous central venous pressure (CVP) measurements, where the placement of central venous catheter was confirmed by appropriate CVP waveforms.

4.4. Haemodynamic monitoring

In each study, measurements were performed with dogs in lateral recumbency and haemodynamic variables were recorded with a multiparametric vital-signs monitor. Heart rate, continuous lead II ECG, invasive systolic, diastolic, and mean systemic arterial pressures (SAP, DAP and MAP, respectively) were recorded. During instrumentation in all three studies, and during the actual investigation period in studies I and II, pulse oximetry, capnography, and ETISO were also recorded. Respiratory rate (RR) was also noted. Body temperatures were monitored with a rectal thermometer, and efforts were made to maintain temperatures by insulating the dogs using a foam mattress and blankets.

Baseline data acquisitions were completed when three consecutive MAP and HR determinations differed by less than 10 mmHg and 5 beats per minute, respectively. In study III after instrumentation, dogs were allowed to recover for a minimum of 60

minutes prior to baseline measurements. Criteria to start the experiment included lack of ataxia and similar level of activity/awareness as before the induction of the anaesthesia.

The cardiac output measurements were performed in studies I and III, using the lithium dilution method (LiDCO Ltd, London, UK). The values were determined by use of a commercial computer and software (LiDCO plus hemodynamic monitor HM 71-02, LiDCO Ltd, London, UK); lithium administration (LiCl dose for each measurement used was 0.005 mmol/kg in study I and 0.075 mmol/dog in study III); and measurements were performed according to manufacturer's instructions, and reports for the use in small animals (Mason et al. 2001; 2002; Beaulieu et al. 2005). Results were corrected a posteriori with each dog's sodium and haemoglobin concentrations taken simultaneously from the arterial catheter for each CO measurement. Cardiac index (CI) values ($mL/m^2/min$) were calculated using CO/BSA, where BSA was calculated from bodyweight and eye to rump length of each individual dog (I). The SVR values were calculated with the formula SVR=80 x (MAP-CVP)/CO (I and III), where an estimated average value of 4 mmHg was used for CVP in the absence of measured values (I).

4.5. Blood gas and other samples

Arterial and venous blood samples were taken from the peripheral artery and jugular vein cannulas. Arterial and venous samples were drawn simultaneously for blood gas analysis at predetermined sampling times in studies I and II. Only arterial blood gas sampling was performed in study III. The samples were placed on ice immediately after sampling, and analysed within 15-30 minutes. Blood gas values were corrected to body temperature. In addition to arterial and venous pH, oxygen and carbon dioxide partial pressures (PO_2 and PCO_2, respectively), haemoglobin oxygen saturation (SO_2), bicarbonate (HCO_3^-), lactate, and haemoglobin (Hb) concentrations were measured. Measurements were performed at 37 °C at sea level standard barometric pressure of 760 mmHg. In addition, during study II the peripheral arterial and central venous oxygen contents (CaO_2 = 1.36 x Hb (g/L) x SaO_2 (%/100) + 0.003 x PaO_2 (mmHg) and CvO_2 = 1.36 x Hb x SvO_2 + 0.003 x PvO_2) and their differences (CaO_2-CvO_2 = a-vO_2 diff) were calculated from the obtained blood gas values after appropriate corrections to obtain canine reference values for oxygen haemoglobin dissociation curve (Reeves et al. 1982).

4.6. Plasma concentration analyses

Venous blood samples were collected for drug concentration analysis at predetermined sampling times from jugular cannulas. In study I, the samples were taken from peripheral jugular cannulas, and in studies II and III the central venous samples were used. Samples were allowed to clot at room temperature for 30-60 min, centrifuged (1000 or 3000 *g*, 20 ^{o}C, 10-15 min), and serum was harvested and stored at maximum -20 C^{o} pending analysis. The specifics of the analyses differed slightly between these studies as the performance and detection of the molecules advanced between the studies. In study I the analyses of MED concentrations were performed with liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI/MS/MS) techniques. In study II and III the separate measurement of plasma DMED, LMED (after chiral separation) and MK-467 concentrations were performed with high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The details of the methodologies during individual studies are available from the original manuscripts (I-III). In study II, plasma concentrations were obtained in a D-optimised blood-sampling schedule and thus different sampling times applied for MED and MK-467 (D'Argenio 1981).

4.7. Sedation scores

In study III, when dogs were not maintained anaesthetised, a composite sedation score (CSS) was obtained by a researcher who remained aware ('non-blinded') of the treatment groups, as the sum of five parameters: resistance to positioning in lateral recumbency, palpebral reflex, position of the eye, jaw and tongue tone, and general appearance at baseline and at predetermined times after drug administration until 60 minutes.

4.8. Analgesia and other drugs

During studies II and III, local infiltration anaesthesia (20 mg of lidocaine 20 mg/mL; Orion, Finland) was used for central venous catheter placement. Meloxicam (0.2 mg/kg Metacam 5 mg/ml; Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany) was administered IV immediately after the last blood sample, and atipamezole (100 µg/kg IM, Antisedan, Orion Pharma, Turku, Finland) was administered if the dogs were still heavily

sedated at the end of the follow-up period (III).

4.9. Statistical and pharmacokinetic analyses

In each study the haemodynamic and blood gas variables were analysed by use of repeated-measures general linear mixed-effect models. All models were built with treatment, time, and the treatment-time interaction as fixed-effect variables, and the animal nested into treatment as a random factor. The covariance matrix structure was individually chosen for each variable based on the best model fit and smallest value of Akaike Information Criterion (AIC). The Kenward-Roger approximation was used to estimate denominator degrees of freedom for repeated measurements. Bonferroni corrections were used to adjust for multiple comparisons as appropriate. Critical level of significance for all comparisons was $\alpha = 0.05$. For sedation scores (discreet values), a generalised linear mixed-effect model with specifications for Poisson distribution was used (III).

Pharmacokinetic analyses were performed from plasma concentration results of studies II and III. From study II plasma concentration results, the individual PK datasets were analysed simultaneously, i.e. population PK analysis (Adapt 5, Pharmacokinetic/Pharmacodynamic System Analysis Software, BMSR, Los Angeles, CA, USA). The model selection and PK parameters were derived with naïve pool data (NPD) analysis, and were used as initial parameter estimates in a population PK analysis (Wang et al. 2009) using maximum-likelihood expectation maximisation (MLEM). The effect of MK-467 on the individual conditional PK parameters was assessed with paired t-tests ($\alpha = 0.05$). The two-compartment model was chosen as it best fitted the data according to the NPD analysis.

From study III, venous concentrations of DMED and LMED and MK-467 were measured at predetermined times between 5 and 120 minutes after drugs administration. Standard PK parameters were initially estimated with non-compartmental analysis (NCA) (Phoenix WinNonlin, version 6.4, Certara, Princeton, NJ, USA) for DMED, LMED, and MK-467. The parameter estimates obtained from the NCA were used as initial values for compartmental methods (Study III) using population PK analysis. The DMED and

LMED fitted a two-compartmental model for extravascular injection, while initial Ka value was estimated graphically using an initial parameter wizard (Phoenix NLME, version 1.3, Certara, Princeton, NJ, USA). This method used First Order Conditional Expectation with interaction (FOCE ELS) with lag time for best fit. For MK-467 a 1-compartmental model was used without lag time using Quasi-Random Parameter Expectation Maximisation (QRPEM) methodology for best fit. The models for each treatment were chosen based on graphically improved fit of model for individual dogs and the lowest Akaike Information Criterion (AIC). The obtained PK parameter estimates were statistically compared between treatments with one-way ANOVA with post-hoc Tukey's test or Friedman's tests with post-hoc Dunn's tests as appropriate were used for statistical comparison with $\alpha = 0.05$. The non-parametric test was used if the data set failed normality. Geometrically right-skewed data was log-transformed prior to statistical testing.

5. Results

5.1. Haemodynamic effects

Under general anaesthesia during MED alone CRI administrations, MAP and SVR (Figure 5.1) initially increased and CI (Figure 5.2) and HR decreased in a dose-dependent manner (I). Similar changes on the measured haemodynamic variables HR, MAP, and CVP associated with MED alone CRI administrations were found in study II (HR shown in Figure 5.3). Similarly, after intramuscular administration in conscious dogs, there was a significant effect of MED on all measured haemodynamic variables (HR, MAP, CVP and CO) (III).

Compared with the baseline value recorded before starting MED administration, CI decreased as MED dose increased for a maximal change of 14.9 (12.7), 21.7 (17.9), 27.1 (13.2), 44.2 (9.7), 47.9 (8.1), and 61.2 (14.1) % (SD), respectively (I). When the different doses were compared, the three lowest dose regimens showed small and short-lived changes in HR, CI, MAP, and SVR, which disappeared before the end of each CRI. Minimal changes were induced by the 0.2 μg/kg/h dose. The maximal effect on SVR and HR with the 0.5 μg/kg/h dose was greater than that of the 1.0 μg/kg/h dose. Generally, the 1.7 μg/kg/h dose resulted in greater effects on hemodynamic variables, in magnitude and duration, than the three lowest doses. The two largest doses showed physiologically and statistically greater effects on each cardiovascular parameter and the duration of these effects was longer when compared to the three lowest doses (Figures 5.1-5.2).

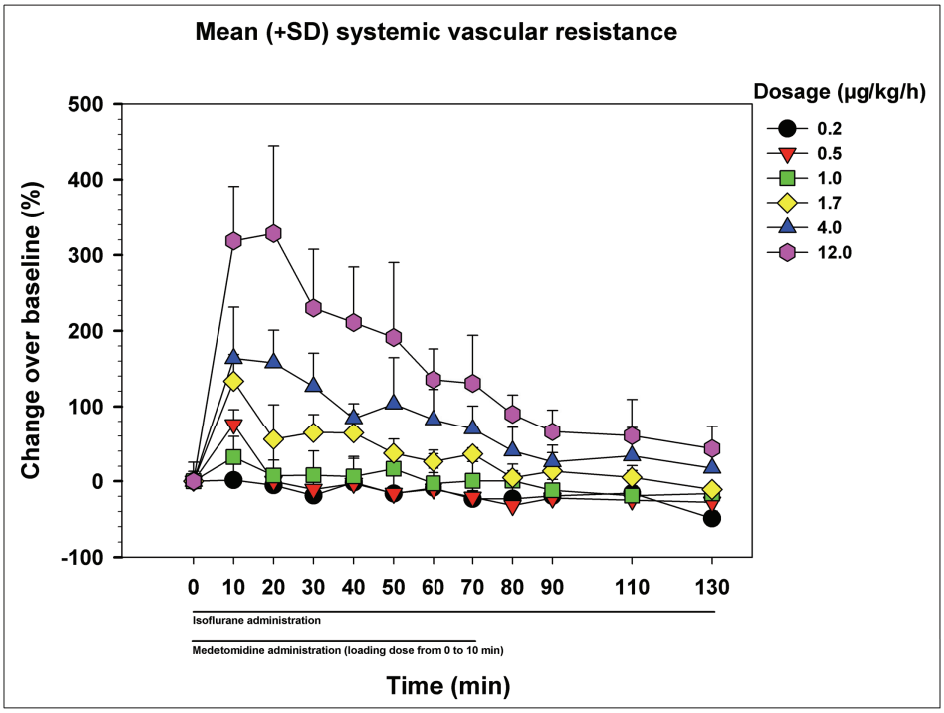

Figure 5.1. – Changes in systemic vascular resistance (SVR) during administration of MED CRI under isoflurane anaesthesia, shown as percentages change (SD) from each group mean baseline values, study I. Time 0 (min) = beginning of MED loading dose administration. Time 10 (min) = beginning of MED CRI. Time 70 (min) = MED CRI administration completed.

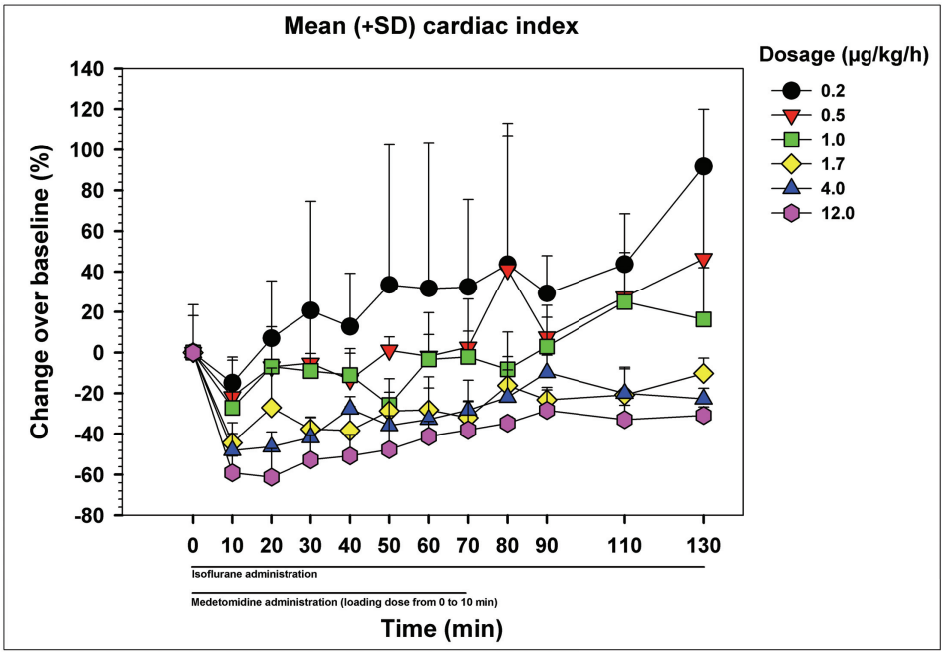

Figure 5.2. – Changes in cardiac index (CI) during administration of MED CRI under isoflurane anaesthesia, shown as percentages change (SD) from each group mean baseline values, study I. Time 0 (min) = beginning of MED loading dose administration. Time 10 (min) = beginning of MED CRI. Time 70 (min) = MED CRI administration completed.

In study II similar haemodynamic changes were reported as in study I and additionally the peak changes in MAP (34.7%) and HR (-32.3%) associated with MED alone were significantly attenuated by addition of MK-467 infusions ($p \leq 0.0015$). Effects on HR are shown (Figure 5.3).

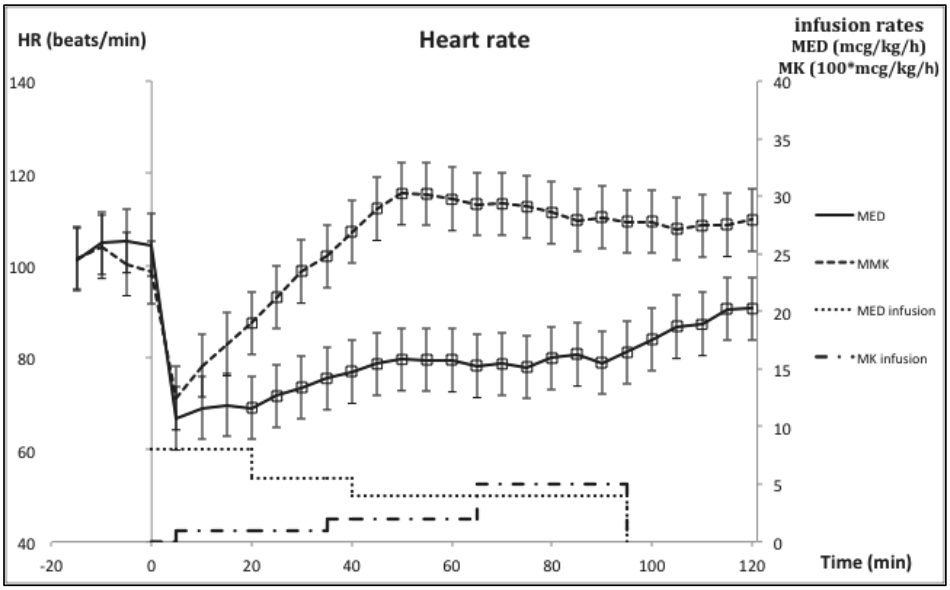

Figure 5.3. – Changes in HR as least-square mean values (95% CI) in dogs receiving intravenous infusions of MED or MMK, study II. The infusion rates are also shown. Empty black squares denote time points that significantly differ between treatments ($p < 0.05$). MED: medetomidine (with placebo); MMK: medetomidine with MK-467.

The effects of intramuscular MK-467 on reversal of MED-induced effects showed dose-dependency on the rate of initiation of the reversal (Study III), which is shown with CO (Figure 5.4). After MED and the lowest dose of MK-467 (MK200) used, it was significantly decreased during the whole 60-minute period, while with the two higher MK-467 doses CO increased back toward baseline and was not significantly different from baseline at 60 (MK400) and 45 minutes (MK600). There were no significant differences between the three doses of MK-467 on the intensity of reversal effects.

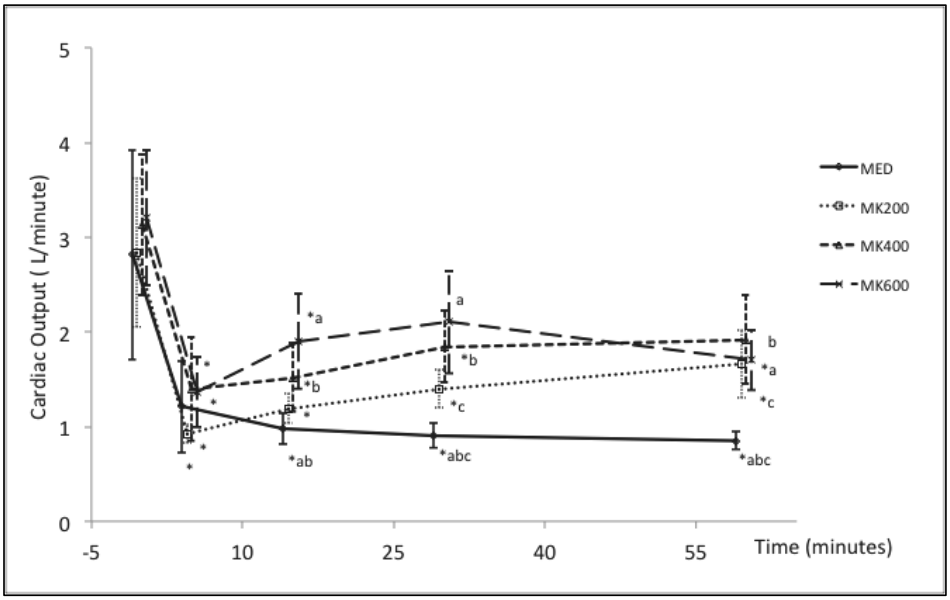

Figure 5.4. – Changes in cardiac output (CO) in eight dogs administered IM medetomidine (20 µg/kg, MED) and its combination with three doses of MK-467 (MK200, MK400, MK600), study III. Values are presented as mean (SD). *significant difference from baseline, [a,b,c]significant difference between treatments with same letter.

The SVR was calculated from the measured values of MAP, CVP, and CO (III). However, in study I, the CVP was not measured and thus it was estimated to be 4 mmHg for the SVR calculations, as it was assumed to have relatively small variation compared to MAP values. It demonstrated significant increase associated with MED administration (Study I: Figure 5.1; Study III: Figure 5.5). The effects associated with MK-467 showed some dose-dependency on the initiation of the reversal but there was no dose relation on the intensity of antagonism (Figure 5.5).

Some dogs showed second-degree atrioventricular blocks after the administration of MED but their duration was usually brief and no treatment was required (I-III).

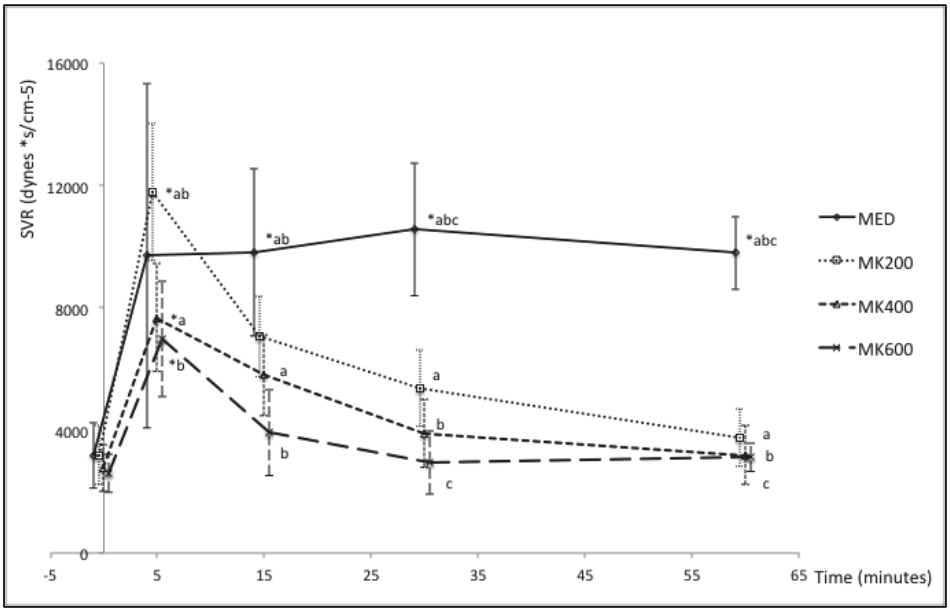

Figure 5.5. – Changes in SVR in eight dogs administered IM medetomidine (20 µg/kg, MED) and its combination with three doses of MK-467 (MK200, MK400, and MK600), study II, but not published before. Values are presented as mean (SD). *significant difference from baseline, [a,b,c]significant difference between treatments with same letter.

5.2. Pharmacokinetic effects

Statistical comparison of the plasma concentrations of MED normalized to the unit dose (1.0 µg/kg/h) revealed significant effects of dose and time ($p < 0.0001$ in both cases), as well as the time x dose interaction ($p = 0.0001$) (Figure 5.6, Study I). It is worth noting that dose-normalized serum MED concentrations during the maintenance CRI appeared to have an increasing slope in the 0.2 and 0.5 µg/kg/h groups, but slightly decreasing slope in the four higher dosage groups. No statistical differences were evident between the four highest-dosage groups ($p > 0.12$). Investigation of the plasma DMED and MK-467 concentrations from study II revealed that the addition of MK infusion to MED infusion decreased the mean DMED plasma concentration (Figure 5.7).

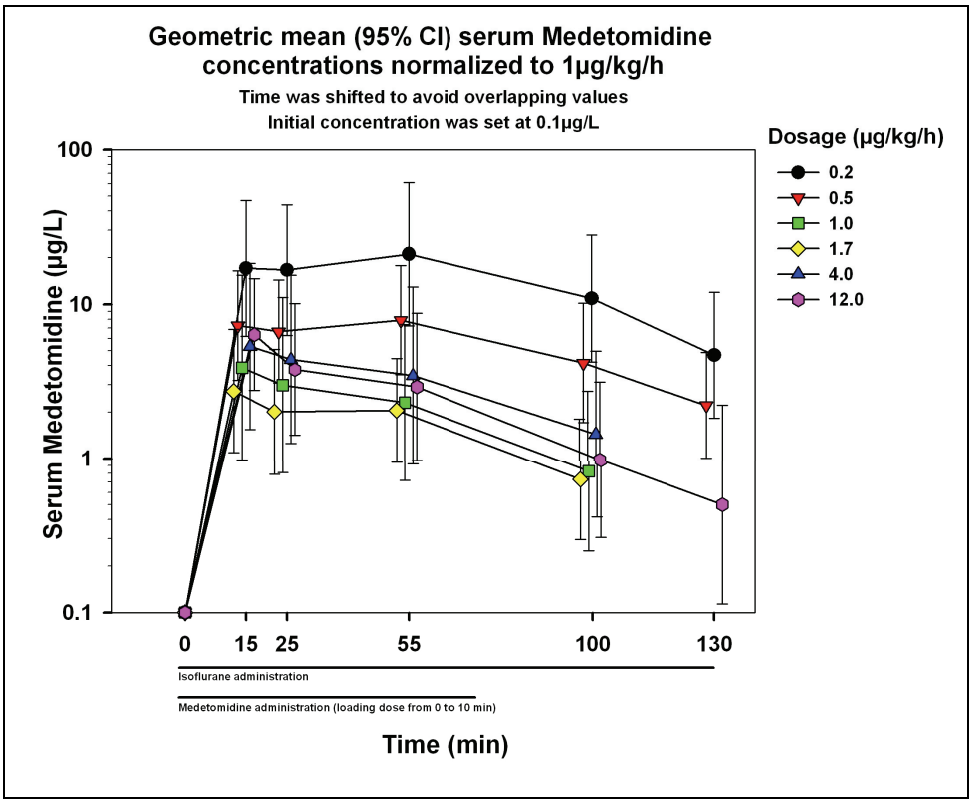

Figure 5.6. – Mean serum MED concentrations (95% CI) for the six infusion rates, normalized to 1.0 µg/kg/h infusion rate, study I. Time 0 (min) = beginning of MED loading dose administration. Time 10 (min) = beginning of MED CRI. Time 70 (min) = MED CRI administration completed. A semi-logarithmic scale was used for presenting the data and all concentrations artificially started from zero as a baseline value.

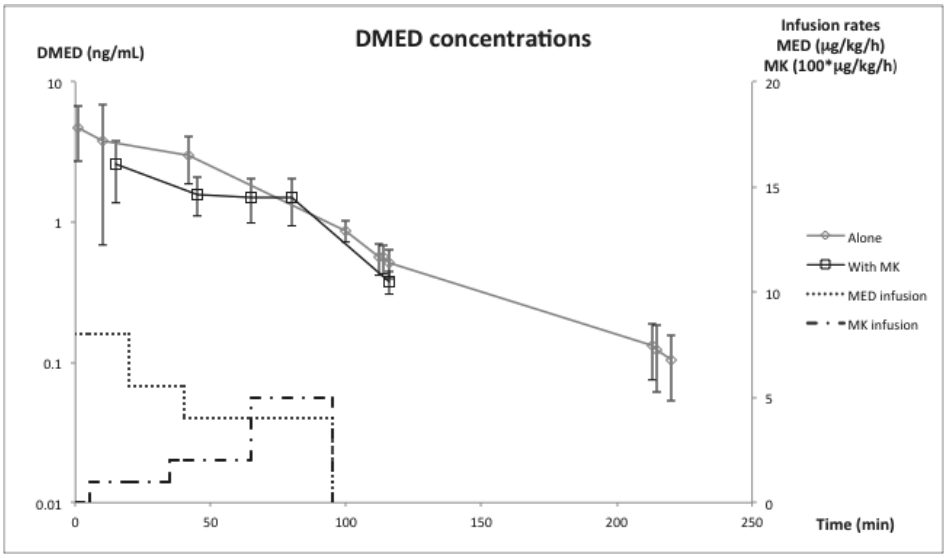

Figure 5.7. – Venous plasma concentrations of the active enantiomer DMED presented as mean values and standard deviations (SD) on a semilogarithmic scale in dogs receiving intravenous infusions of MED alone or with MK, study II. The infusion rates are also shown.

The plasma concentration data from study II showed that the two-compartment pharmacokinetic model best fit this data. The maximum likelihood expectation maximisation (MLEM) method showed that only the systemic clearance (CLt = 0.0359 L/kg/min; residual standard error = 11.8%) differed across treatments (p = 0.0008): it was 24.1% higher in the MMK period [Figure 5.8, (Kaartinen et al. 2014), abstract]. The addition of MK-467 failed to alter the DMED distribution clearance and the volumes of distribution. The inter-individual coefficients of variation (CV) of DMED PK parameters were maximally 36%.

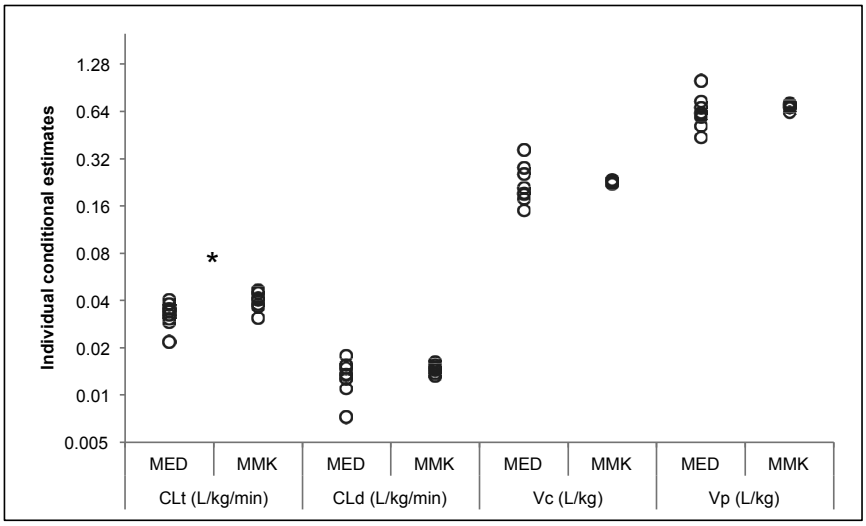

Figure 5.8. – DMED systemic (CLt) and distribution (CLd) clearances, volumes of the central (Vc) and peripheral (Vp) compartments in dogs in presence (MMK) or absence (MED) of MK-467 (*, significant effect), [(Kaartinen et al. 2014), abstract].

The raw data of the plasma concentrations of DMED and MK-467 after IM co-administrations (III) are shown in Figures 5.9 and 5.10, respectively.

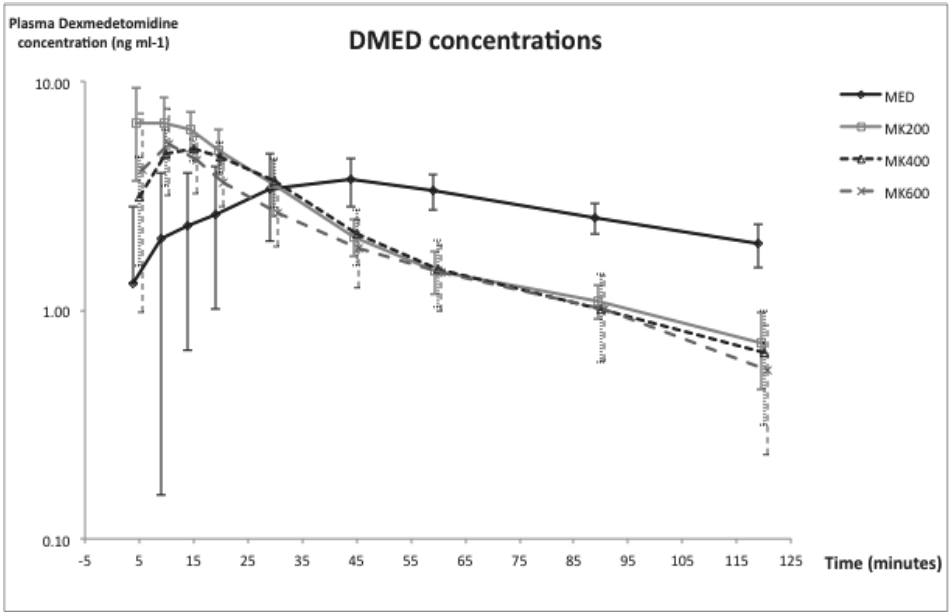

Figure 5.9. – Venous plasma concentrations of the active enantiomer DMED presented as mean values and standard deviations (SD) on a semilogarithmic scale in dogs receiving IM MED alone (20 µg/kg, MED) and its combination with three doses of MK-467 (MK200, MK400, and MK600), study III.

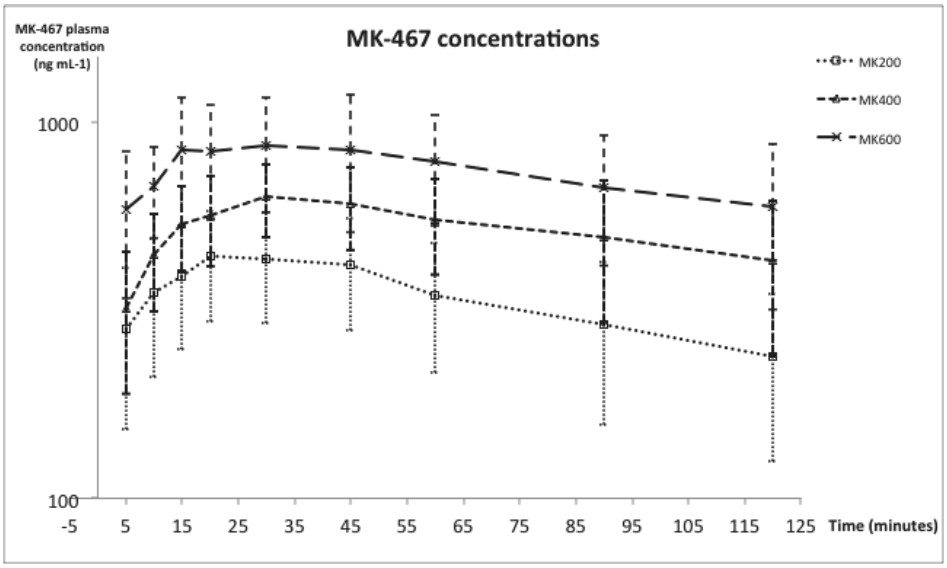

Figure 5.10. – Venous plasma concentrations of MK-467 presented as mean values and standard deviations (SD) on a semilogarithmic scale in dogs receiving IM MK-467 at 3 doses (MK200, MK400, and MK600) in combination with MED (20 µg/kg), study III.

Pharmacokinetic investigation of the plasma concentrations of DMED (Figure 5.9) and MK-467 (Figure 5.10) after concomitant IM administration (Study III) showed, that the MK-467 significantly accelerated the rate of the active enantiomer DMED absorption (significantly higher C_{max} and earlier T_{max}; Figure 5.9). These results revealed the effects of MK-467 on MED pharmacokinetics lacking dose-proportionality. Summaries of the results from the population PK analysis that best fitted a two-compartmental method for DMED and LMED are presented in Table 5.1 and one-compartmental method for MK-467, presented in Table 5.2. These results indicated that MK-467 increased the absorption of both DMED and LMED and increased clearance from the central compartment. The volume of distribution of the central compartment (both DMED and LMED) was significantly affected only with the lowest dose of MK-467. These results revealed no effect of MED on MK-467 kinetics. When the inter-individual coefficients of variation (CV) are above 30% this corresponds to relatively high variability, which indicate poor estimations of those parameters by the model.

Table 5.1. Population PK analysis parameter estimates for DMED and LMED after MED (20 μg/kg IM) alone or with MK-467 (3 doses; 200, 400, and 600 μg/kg IM), study III.

	DMED				**LMED**			
	Abs. $T_{1/2}$ (min)	V_1 (L/kg)	Cl_1 (mL/min/ kg)	t_{lag} (min)	Abs. $T_{1/2}$ (min)	V_1 (L/kg)	Cl_1 (mL/min /kg)	t_{lag} (min)
MED20	13.8	1.6	20.3	2.0	12.6	2.0	27.5#	2.0
CV (%)	30.2	1.52	10.6	69.8	61.5	256.2	29.2	55.2
MMK200	2.3*	0.7*	31.1*	1.3	3.2*	0.5*	37.8*#	1.1
CV (%)	49.4	22.9	11.8	51.7	54.4	29.9	7.0	58.2
MMK400	5.9*	0.9	32.2*	1.7	6.0*	1.4#	34.6*	1.6
CV (%)	22.7	5.7	16.7	39.7	19.8	5.2	21.6	42.8
MMK600	3.8*	0.8	39.0*	2.9	4.0*	1.2#	46.2*#	2.7
CV (%)	30.9	26.3	9.4	8.7	31.6	23.3	10.5	24.1

*Significant difference from MED20, #Significant difference from DMED. Abs. $T_{1/2}$: Absorption half-life, V_1: Volume of distribution to central compartment, Cl_1: Clearance from central compartment, t_{lag}: lag time, CV: coefficient of variation.

Table 5.2. Population PK analysis parameter estimates for MK-467 after administration in combination with MED (20 μg/kg IM) and MK-467 (200, 400, and 600 μg/kg IM), study III.

	MK-467		
	Abs. $T_{1/2}$ (min)	V (L/kg)	Cl (mL/min/ kg)
MMK200	5.1	0.4	2.9
CV (%)	36.7	14.0	24.8
MMK400	6.6	0.6	2.4
CV (%)	17.4	13.2	56.3
MMK600	4.56	0.63	2.5
CV (%)	38.1	20.0	51.7

Abs. $T_{1/2}$: Absorption half-life, V: Volume of distribution, Cl: Clearance, CV: co-efficient of variation.

5.3. Other effects

5.3.1 Blood gas and respiratory variables

The effect of MED on arterial or venous pH is dependent on the dose given (I). The arterial pH of some dogs dosed with the two highest MED infusions decreased below the reference interval (7.35 - 7.45), a difference that reached statistical significance with the highest dose (I). With the addition of MK-467 to the MED infusion the pH increased significantly compared to MED infusion alone during which pH remained in a lower range (II). After IM administration of MED and MK-467, no differences were detected between treatments in arterial pH or bicarbonate concentration, although the values showed high inter-individual variability (III).

The other measured respiratory, metabolic, and tissue perfusion variables (glucose, HCO_3^-, $PaCO_2$, or lactate) stayed within a physiologically normal range during the different dose MED infusions in arterial and venous samples (I). When venous values were compared between MED and MED+MK infusions, there was a significant effect on $PvCO_2$ ($p \leq 0.0073$). The $PvCO_2$ was higher during MED alone than during MED+MK infusion (II). The significant differences between treatments occurred at the two highest MK infusion step rates. In conscious animals the addition of MK-467 significantly increased $PaCO_2$ while lactate values remained stable (III). However, the $PaCO_2$ values remained in a physiological range during all treatments.

Arterial oxygen tension (PaO_2) was maintained during all infusion dose regimens (I) while a high concentration of oxygen was provided to administer isoflurane. Venous oxygen tension (PvO_2) decreased significantly with the three highest doses, with a dose-dependent tendency. When venous values were compared between MED and MED+MK infusions (II), there was a significant effect on Hb ($p \leq 0.0018$), SvO_2 ($p < 0.0001$), and PvO_2 ($p \leq 0.0055$). The venous oxygen saturation and tension (SvO_2 and PvO_2) values were significantly lower during MED infusion than during MED+MK infusion at the two highest MK-infusion step rates. In addition, the arterio-venous oxygen content difference (a-vO_2 diff) showed peak changes of 222.5% associated with MED alone and it was significantly attenuated by MK-467 ($P \leq 0.0015$): maximal attenuation relative to peak changes was 65.4% (Figure 5.11). In conscious animals the PaO_2 stayed stable (III).

In conscious dogs, the respiratory rates were significantly lower than baseline from 10 minutes onward with all doses of MK-467, but no differences were detected between these treatments (III).

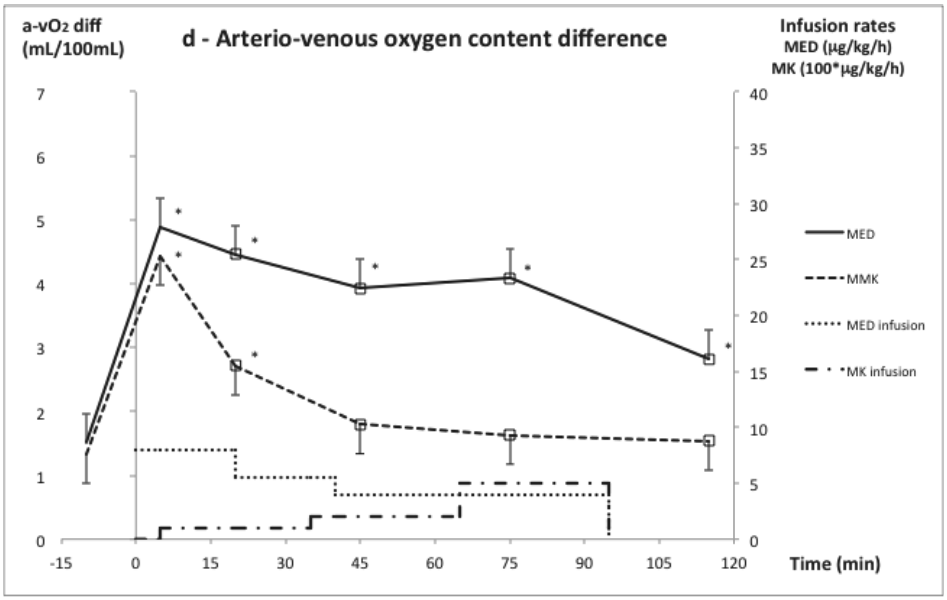

Figure 5.11. – Changes in arterio-venous oxygen content difference as least-square mean values and 95% confidence intervals in dogs receiving intravenous infusions of MED or MMK, study II. The infusion rates are also shown. Empty black squares denote time points that significantly differ between treatments ($p < 0.05$) and *significant difference from baseline. MED: medetomidine (with placebo); MMK: medetomidine with MK-467.

5.3.2. Sedation

In study III all the dogs were clinically sedated during all treatments, and the composite sedation scores increased significantly from baseline (Table 5.3). Within treatments MK200 and MK600, the sedation scores increased significantly earlier, and later MK-467 shortened the duration of sedation with all given doses, compared to MED alone. With addition of MK-467, the maximum scores were reached within 15 minutes while with MED alone it appeared to take longer. The sedation scores decreased with MK-467 during the 60-minute follow-up period, while with MED alone the sedation scores remained at the maximal level.

Table 5.3. Composite sedation scores (CSS) in dogs administered medetomidine (20 µg/kg, MED) and its combination with three doses of MK-467 (MK200, MK400, and MK600), study III. Values are shown as median (min;max).

	Baseline	5 min	15 min	30 min	60 min
CSS (0-16)					
MED	0 (0;3)	6.5 (1;10)*[a]	12 (8;16)*[a,b]	13.5 (10;16)*	14 (11;16)*[a,b,c]
MK200	0 (0;3)	9 (9;13)*[a]	14.5 (13;16)*[a]	15 (11;16)*	9 (8;13)*[a]
MK400	0 (0;4)	8 (4;13)*	14 (11;15)*	13 (7;16)*	11 (8;14)*[b]
MK600	0 (0;3)	8.5 (1;13)*	15 (12;16)*[b]	14.5 (8;16)*	9.5 (3;13)*[c]

*significant difference from baseline, [a, b, c]same letter = significant difference between treatments.

6. Discussion

6.1. Haemodynamic effects

This investigation describes the quantitative documentation of the dose-dependency of MED effects on MAP, SVR, CI, and HR (I). Typical alpha-2 agonist-related increases in blood pressures and SVR with associated decreases in HR and CI were observed, where intensities and durations depended on the administered CRI dose. The findings on the dose-dependency of the degree of cardiovascular effects of MED in anaesthetised dogs are comparable to previous dose-titration studies in conscious dogs (Pypendop & Verstegen 1998; Carter et al. 2010). However, our dogs anaesthetised with isoflurane, demonstrated lower baseline values compared to conscious dogs. Consequently, the initial vasopressor effect of MED was more evident in anaesthetised dogs, allowing differentiation of the dose-response relationship. Moreover, our administration modality was a CRI as described in the study by Carter et al. (2010) and in contrast to an IV bolus administration used by Pypendop & Verstegen (1998), which permitted our study to yield slower changes in plasma concentrations and consequent cardiovascular effects. Furthermore, our results are in accordance with results described for DMED infusions in isoflurane-anaesthetised dogs, although the isoflurane concentration administered was changed in this investigation in order to quantify the MAC sparing effects of DMED (Pascoe et al. 2006).

The hemodynamic effects were of a lesser intensity and shorter duration with the three lowest MED doses, disappearing before the end of each CRI. They were greater and longer in duration with the middle dose and most pronounced with the two highest doses investigated. These results showed that the effects from loading doses were important and thus smaller loading doses could be appropriate to assess the effects from CRI doses. As several small dose increments were investigated, the intensity of effects appeared, in some instances, to be greater for smaller doses than the next (higher) dose used, for example in the case of SVR and HR, implying a too-narrow dose range alteration relative to high inter-individual variation, and small sample size, making it statistically improbable to differentiate between the adjacent dose levels. However, the overall results clearly demonstrated the dose-dependency of the investigated haemodynamic effects of

MED. Consistent with the previous dose titration study (Pypendop & Verstegen 1998), the duration of these changes became longer when increasing the dose (I).

The MK-467 infusion rapidly began antagonising the haemodynamic effects of MED when these effects were already present and while MED was infused at the highest rate (MED:MK dose ratio as high as 1:12.5, during which DMED:MK plasma concentration ratio approximately 1:50) (II). Reduction of the MED:MK dose ratio further attenuated MED-induced effects. The step infusion design enabled the determination of whether maximal effect was reached or if further change could be achieved by altering the dose ratio. These haemodynamic findings were consistent with, and further supplemented the results from, previous single-dose administration reports on MED–MK or DMED–MK interaction in dogs (Enouri et al. 2008; Honkavaara et al. 2008; Honkavaara et al. 2011; Rolfe et al. 2012).

The dosage rationale for the step infusion of MED was based on estimates from earlier results (Kuusela et al. 2000) and on previous knowledge gained from study I. The step infusion design was inspired by Bailey (1991). Briefly, based on study I, the loading doses were potentially higher than adequate for the CRI doses, thus a smaller loading dose was chosen for MED. As the most appropriate doses of MED from study I were 1 and 1.7 µg/kg/h without MK-467, higher plasma levels than achieved by these were sought, in order to see the full effects before antagonist was initiated. The exact dose rates were estimated for rapidly achieving a pseudo steady state for MED. No loading dose was used for MK-467 as small increments of the antagonist were anticipated to elicit dynamic changes. This design would facilitate the evaluation of these effects from initiation and as they slowly increased, rather than obtaining full reversal. The durations of each infusion step were based on calculated estimates of pharmacokinetic parameters combined from previously published results (Kuusela et al. 2000; Honkavaara et al. 2012) and from unpublished preliminary results on DMED and MK-467 plasma concentrations from our research group.

Intramuscular administration of MED and MK-467 showed a dynamic interaction, the interpretation of which has proven challenging: MK-467 attenuated the cardiovascular effects of MED only after the initial absorption phase, but increasing the MK-467 dose failed to significantly change the intensity of these effects (III). However,

there was a MK-467 dose-related trend to decrease the duration of MED effects reflected by significant differences at later follow-up between combination treatments and MED alone. Moreover, the initial cardiovascular effects of MED during the absorption phase were not prevented or attenuated by simultaneous antagonist administration.

In line with all our cardiovascular findings, the effects of DMED have been previously suggested to depend on the initial status of blood vessel tone (Lin et al. 2008). In addition, it has been suggested that the central sympatholytic effects may predominate at low doses, stimulating the central alpha-2A adrenergic receptor subtype, the source of sympatholytic effect, sedation, and analgesia (Kamibayashi & Maze 2000). At higher or rapidly-injected doses, however, greater peripheral vasopressor effects arise, which result from stimulation of the vascular alpha-2B receptors (Aantaa & Jalonen 2006). In agreement with previous studies on MED in dogs (Kuusela et al. 2003), no hypotension was subsequently observed (I). The magnitude of change in the middle MED CRI dose (1.7 µg/kg/h) used was comparable with results associated to a similar dose of MED (1.5 µg/kg/h) leading to decrease in HR (-41.7%) and CI (-41.2%) (Grimm et al. 2005). The data in study I are also consistent with previously-reported results with corresponding doses of DMED (Flacke et al. 1993; Pascoe et al. 2006; Lin et al. 2008). In study II, blood pressures increased during MED administration as expected based on study I, followed by a significant decrease when MK-467 infusion rate was increased, during which time the MED:MK ratio was between 1:18 and 1:50. At the third infusion step, when the MED:MK ratio was lowest (1:125), the mean MAP decreased below 60 mmHg, which is a generally-accepted limit for hypotension and maintaining the vital organ perfusion pressures (Moens & Coppens 2007). This hypotension is consistent with the reported effects of MK-467 administered alone in rats (Szemeredi et al. 1989), but has not been reported by others in conscious dogs (Pagel et al. 1998; Honkavaara et al. 2011). In addition, during general anaesthesia, an IV bolus administration of MED:MK dose ratio of 1:25 as a premedication prior to propofol and isoflurane administration maintained normal blood pressures (Salla et al. 2014a). Thus, based on our results, the lowest MED:MK dose ratio (1:125), corresponding to DMED:MK plasma concentration ratios of approximately 1:1000, cannot be recommended, at least in dogs under isoflurane anaesthesia. It is worth noting that as the peripheral vasopressor effects of MED CRI

differ in dogs under anaesthesia (Study I) compared to conscious dogs (Carter et al. 2010), the effects of MK-467 may differ as well.

From the results in study III, it was evident that IM MK-467 failed to immediately antagonize the effects of MED. In fact, CVP and MAP appear initially more pronounced with MK-467 during the highest DMED plasma concentrations, especially by the lowest MK-467 dose, although no statistical difference was evident. The antagonist effects of MK-467 appeared later than the effects of MED probably due to the slower absorption rate of MK-467 than DMED. Moreover, the plasma concentration results supported the haemodynamic findings. For example, at five minutes after the lowest dose of MK-467 (200 µg/kg), the DMED:MK concentration ratio in plasma was high (approximately 1:40), but later when the ratio decreased (e.g. more MK-467 was absorbed and increased its plasma levels) the cardiovascular effects of MED were also reversed. This finding was consistent with study II, where during the first infusion step of MK-467, the plasma concentration ratio (DMED:MK ratio approximately 1:50) failed to fully reverse the cardiovascular effects of MED during IV infusions.

The peripheral vasoconstriction resulting from alpha-2B receptor stimulation induced an increase in SVR that was very limited in intensity and duration at the lower MED CRI doses (I). This pharmacological action governs the increase in MAP and contributes to bradycardia, leading to reduced CI. Particularly evident at the low doses, HR was reduced for a longer time in comparison to other haemodynamic variables, as demonstrated in a previous report, where HR decrease also persisted at low serum concentrations in dogs (Kuusela et al. 2000). This could be related to the sympatholytic effects via central alpha-2A adrenoceptors (Maze & Fujinaga 2000). Interestingly, MK-467 attenuated the other haemodynamic effects of MED more readily than the arterial pressures: the effects on HR, and CVP were already evident at the lowest dose rate of MK-467, when the MED:MK ratios were from 1:12.5 to 1:18 and DMED:MK plasma concentration ratios varied from approximately 1:50 towards lower ratios (II).

Thus, although the haemodynamic effects of MED and the reversal of these effects by MK-467 are clearly demonstrated in all our studies, it remains difficult to suggest what the optimal doses and dose ratios are, as some variables appear to be more readily affected than others. In addition, based on study III, the IM administration of MK-

467 could potentially prevent most vascular effects of MED IM if it would be administered 5 to 10 minutes prior to MED. Further, PK and PD modelling are required in order to estimate the relative potency of MK-467 on all the haemodynamic variables. These analyses would improve the determination of the optimal MED:MK ratio.

These results have some potential limitations. Study I showed great inter-individual variation on the haemodynamic and plasma concentration results. Thus although this study provides preliminary knowledge on these variables, the sample size of four dogs per group is too small to appropriately quantify these effects. In all studies, the monitors used for analysing expired isoflurane concentration were not calibrated for the experiments. In addition, the invasive blood pressure transducers were zeroed to the atmosphere, but they were not calibrated for experiments. In study II, two different sites were used for arterial cannulas, to preserve the commonly-used pedal dorsal arteries. Cannulas were placed either in the auricular or tail artery, and there are potential differences in the blood pressure waveforms between these sites. However, similar number of dogs in both groups had blood pressures measured from auricular arteries and thus this error was presumably similar in each group. In studies I and III, where CO was measured by lithium dilution methodology, recent evidence suggests that the investigated drugs, including all alpha-2 agonists, may affect the lithium sensor voltage *in vitro*, although only xylazine appears to be able to alter the results at clinically relevant concentrations *in vivo* (Ambrisko et al. 2013; Ambrisko & Moens 2014). In study II, CO was not measured in order to increase the quantity of blood available for plasma concentration samples, as lithium dilution method requires sampling of blood across the sensor during each measurement. Thus, this study lacked CO measurements in order to gain more knowledge on the plasma concentrations and pharmacokinetics. However, as the plasma concentration samples were taken based on D-optimised sampling schedules that differed between MED and MK-467 sampling times, no simultaneous concentrations of both MED and MK-467 were obtained, which would have been more useful in order to have better judgement on the actual plasma concentration ratios during different dose ratios given.

6.2. Pharmacokinetic effects

The four higher MED CRI rates demonstrated homogenous pharmacokinetics, while the two lowest doses presented a different pharmacokinetic pattern (I). Specifically, the lower the rate below 1.0 μg/kg/h, the greater appeared to be the dose-normalized plasma concentration of MED. This was further evidenced with the difference in elimination slopes upon completion of the initial loading dose between the four highest and the two lowest dosage groups. This difference in dose-normalised MED concentrations may suggest that MED systemic clearance increased with dose from ultra-low dose toward higher doses, and reached a plateau. Alternatively, this may be a demonstration of the lower loading doses revealing a distribution phase instead of plateau during CRI administration. In contrast, the higher loading doses revealed more marked effects on CO, which may have slowed their own elimination rate as has been previously suggested (Dutta et al. 2000; Pypendop et al. 2013). This has been further evidenced in more recent studies with the addition of MK-467 effectively increasing CO (or attenuating the decrease induced by MED or DMED) and increasing MED elimination (Honkavaara et al. 2012; Bennett et al. 2016b), similarly shown in our findings from study II and III. Furthermore, the metabolism of MED may have different pathways and kinetics during different dose rates. The metabolism of MED appears mainly (80-90%) by hepatic hydroxylation followed by glucuronidation in dogs involving several biotransformation pathways (Salonen 1992). Different enzymes accomplish the phase II glucuronidation of MED, with different affinity, regio-, and stereoselectivity in human and canine hepatic microsomes leading to N-glucuronidation of DMED and LMED with different kinetics (Kaivosaari et al 2008). In addition, a relatively low enzyme saturation and hepatic clearance was shown in canine hepatic microsomes, that may limit the rate of MED metabolism (Duhamel et al. 2010). In study I, the isoflurane level was kept similar between the MED doses, which might have affected the results as isoflurane level was not decreased to account for the dose-dependent anaesthetic sparing effect of MED. The higher anaesthetic depth could have increased the plasma concentrations measured, by further reducing the hepatic and kidney blood flow, which would further decrease the elimination of MED. However, without measuring the individual MAC and quantifying the MAC sparing of each MED CRI dose, this would have been difficult to perform, thus

a lowest level of isoflurane that was adequate to maintain dogs lightly anaesthetised without MED CRIs was used during all treatments.

Exploratory analysis of the plasma concentrations against time of DMED and MK during step infusions revealed that plasma DMED concentrations appeared reduced during MK administration, although without reaching statistical significance (II), similar to previous reports for DMED and MED single IV dose administration (Honkavaara et al. 2012; Bennett et al. 2016b). In our MED-MK infusion study (II), a pharmacokinetic step infusion procedure inspired from Bailey (1991) was used in order to rapidly achieve and maintain constant plasma concentrations of MED. We showed that relatively stable concentrations could be achieved rapidly, but the plasma MED concentrations decreased over time during the step-infusions of the MED-alone. This suggests that our initial parameters that were estimated from the reported MED pharmacokinetic parameters (Kuusela et al. 2000) slightly underestimated the actual decay of plasma MED concentrations during the distribution phase in our dogs. Nevertheless, we believe that this decrease was sufficiently shallow to produce valid interpretation of the results. In addition, the step infusion approach enabled a range of effective MK-467 levels below maximal effect (E_{max}), thus demonstrating dose-effect by increasing the MK-467 concentration with each further step during stable MED concentrations.

Plasma DMED concentrations were subsequently analysed with population PK analysis and the apparent decrease in DMED concentrations revealed during initial exploration was confirmed to be a significant effect of MK-467 on the systemic clearance of DMED. These results suggest that MK-467 only affects MED elimination with the concentrations attained (II) and in addition, revealed increases in effects that followed each change in MK infusion rate, an indication that our dosing regimen progressively approached E_{max}. The step-infusion design was successful toward the aim to find the range of MED:MK dose ratios between 1:18 to 1:50, during the second step of infusions, that would include the optimal ratio for clinical use.

The intramuscular route of administration revealed that an interaction of MED and MK-467 also appears during absorption from the site of administration (III). Most previous investigations have concentrated on intravenous administration, which bypasses the absorption phase. In our study, MK-467 initially facilitated the absorption of MED,

when both drugs were administered in the same syringe, reflected by higher and more-rapidly reached maximum plasma concentrations of DMED and LMED, and faster absorption constants. Our study was limited as the duration of blood sampling only advanced until 120 minutes after drug administration. Therefore, these data did not allow for good estimates of the elimination phase of either MED or MK-467 and hence the main findings concentrated on absorption kinetics and the corresponding initial effects following drugs administration. This study was designed to investigate effects during a clinically-relevant time period, however, thus a short follow-up was considered acceptable. To extend our knowledge, further studies using a meta-analysis from several study populations analysed concomitantly, including longer sampling periods, will allow for better estimates also during the elimination phase.

A pharmacokinetic drug interaction may explain the pharmacodynamic alterations in the results (III). This is in contrast with a previous report which suggested that after an IM injection, the absorption and/or distribution of MK-467 was faster or similar to MED alone, as the concurrently-administered MK-467 IM immediately attenuated the systemic peripheral effects of MED IM (Rolfe et al. 2012). Nevertheless, these authors failed to report plasma drug concentrations to confirm their suggestion. The doses of MED and MK-467 used in that study were similar to the middle dose used in our study (MK-467 400 μg/kg). However, they administered MED in a separate syringe immediately followed by MK-467 injection in the lumbar muscle group, whereas we mixed MED and MK-467 in the same syringe. Rolfe et al. (2012) did not specify whether different injection sites were used, and also the dilution of their drugs was higher. In addition, the manufacturers of MK-467 differed between the studies. In our study, MK-467 may have prevented the local peripheral vasoconstriction caused by MED, thus facilitating the absorption of MED at the injection site. The pharmacokinetic analyses confirmed that the addition of MK-467 to IM MED hastened the absorption and elimination of MED. The absorption appeared highest with the smallest dose of MK-467, however, there were no significant differences between MED absorption half-lives between the three doses of MK-467 used. Thus, the dose dependency of these pharmacokinetic alterations could not be demonstrated with the three investigated MK-467 doses (III).

In all three studies, venous plasma concentrations were measured instead of arterial concentrations. In study I, the jugular venous plasma samples were obtained and may be viewed as peripheral samples, although the distance to the central venous site may be negligible. In the two latter studies (II-III), the central venous plasma samples were obtained. There are arteriovenous differences in plasma concentrations, and commonly after IV infusion, arterial concentrations are initially higher compared to venous, the elimination curves parallel but venous concentrations are slightly higher during elimination (Olofsen et al. 2010). If venous concentrations are used to estimate pharmacokinetic and -dynamic parameters, this may result in an underestimation of drug decay and overestimation of its potency. This difference is considerable when there are differences in distribution and elimination between arterial and venous sites (Gumbleton et al. 1994), and the differences are usually more important if peripheral (arm or leg) vein of poorly perfused tissue is used for blood sampling. The differences between venous and arterial drug concentrations are mainly due to a high efficiency of drug uptake by the relatively poorly-perfused peripheral sampling tissue (arm or leg) during its very short transit through the capillary (Chiou 1989). Nevertheless, some studies consider both central venous and arterial concentrations to be representative of central compartment drug concentrations; particularly during long-term drug infusions and thus either sample may be deemed appropriate (Iirola et al. 2012). Venous concentrations were used in all the studies described here for ease of comparison between these investigations, and considered that these jugular and central venous sampling sites would satisfactorily estimate concentrations from the central compartment. However, the potential differences between these results compared to others using arterial sampling sites should be taken into consideration.

6.3. Other effects

6.3.1. Blood gas and respiratory variables

A metabolic acidosis has been reported in earlier studies with MED and DMED (Kuusela et al. 2001a; Uilenreef et al. 2008), and was sporadically found at the highest MED CRI dose rates under investigation (I). In addition, during the MED-MK step infusion study

(II), when MED dose rate was highest and MK-467 lowest, there was a mild metabolic acidosis that was reversed by increasing the MK-467 dose rates. Otherwise, both venous and arterial pH, carbon dioxide tensions (PCO_2), and bicarbonate concentrations remained within the physiological range during each dose of MED CRIs (I), and were consistent with previous findings (Pascoe et al. 2006; Lin et al. 2008). Since lactate-free, high-chloride-content fluids were administered in our study, similar to the study from Uilenreef et al. (2008), the possibility of hyperchloremic acidosis was verified and no changes in chloride concentrations were found (data not published). Further, in study I, acidosis was apparent only with higher doses, which also argues against fluid-induced acidosis, and supports the dose-dependent effect of MED. However, this study was limited to blood gas measurements not extending beyond the MED administration period. Furthermore, based on the previous report, the magnitude of this acidosis is not clinically relevant in healthy dogs (Kuusela et al. 2001a). Our study I revealed a dose-dependent effect on pH, which has not been shown in earlier studies. The results indicate a further advantage of decreasing the MED CRI dose below the level of potential metabolic acidosis (I) or addition of MK-467 to the treatment (II).

The results from study II demonstrated that $PvCO_2$ was lower during combined administration of MED and MK-467 than during MED in dogs under controlled ventilation. Although the mechanism of this effect is currently unclear, there appears to be an increase in $PvCO_2$ during MED administration that is subsequently countered by MK-467. It may be suggested that with MED the decreased peripheral blood flow could increase the time for gas exchange in tissues, increase $PvCO_2$, and decrease PvO_2 and pH, the effects that may be reversed by MK-467.

In conscious dogs (II), after IM administration of MED and MK-467 there was a slight but significant decrease in respiratory rate and increase in $PaCO_2$ with MK-467 in addition to MED, however no clinical change in alveolar ventilation was detected, as $PaCO_2$ remained within a physiologically acceptable range (Ilkiw et al. 1991).

Arterial lactate concentrations remained low and revealed similar findings reported previously in conscious dogs (Honkavaara et al. 2011; Restitutti et al. 2012) as well as with DMED during anaesthesia (Pascoe et al. 2006; Uilenreef et al. 2008), implying that the overall tissue perfusion was maintained during all studies (I-III).

However, the lactate measurement was discontinued after a short follow-up period, and thus potential lactate retention may have occurred. Based on a previous study, some lactate retention may occur when increasing the dose of DMED CRI to 3 μg/kg/h (Uilenreef et al. 2008) but it was not demonstrated with lower doses. Thus, it may be speculated that no lactate retention should occur with low dose MED CRI, or if MK-467 was added to the treatment.

Arterial oxygen tension (PaO_2) during almost 100 % oxygen administration was expectedly high at all MED dose regimens (I). Venous oxygen tension (PvO_2) decreased significantly during the administration period of the three highest doses and this decrease had a dose-dependent tendency but no statistically significant differences between the doses. Similarly, the step-infusion of MED decreased PvO_2 (presumably in association with increased oxygen tissue extraction) and SvO_2, and these decreases were counteracted by MK-467 administration (II). In the conscious animals, PaO_2 remained within normal range, while venous values were not measured (III). These findings imply increased oxygen extraction, especially during higher MED doses, which are consistent with a prior report with DMED (Lin et al. 2008). The underlying cause may be due to a decreased CI and peripheral blood flow. In the two studies on anaesthetised dogs either no central venous catheter was placed (I) or no CO was measured (II). Thus, central venous blood samples or CO measurements were unavailable and oxygen delivery and consumption could not be calculated accurately. However, based on the surrogate results available from peripheral samples (I), and indirect variables calculated as the arterio-venous oxygen content difference (a-vO_2 diff) between peripheral arterial and central venous blood samples (II), the extraction of oxygen appears to increase dose-dependently (I), which has not been reported previously with MED or DMED. The MK-467 attenuated this effect (II). Even though the oxygen balance would remain positive with increasing DMED or MED CRI doses, while oxygen extraction is increased (Lin et al. 2008), these results indicate a further advantage of the use for low dose MED CRI when compared with higher doses (I) and/or addition of MK-467 to the treatment (II). While using jugular venous samples (I), or central venous samples (II), instead of true mixed venous samples to calculate a-vO_2 diff may introduce a source of error when estimating oxygen extraction, the usefulness of central venous oxygen saturation values in the

absence of mixed venous values has been validated (Rivers et al. 2001) and in addition the same error would presumably have been introduced with each treatment.

During study I, no attempts were made to correct the blood sample oxygen saturation and Hb content values to follow a canine oxygen haemoglobin dissociation curve. During studies II and III, these values (SaO_2, SvO_2, Hb) and calculated parameters using these values (CaO_2, CvO_2, a-vO_2 diff) were corrected (Reeves et al. 1982). Thus, these results from study I may not reflect accurate values for the canine population. However, as the same error would presumably be present in all groups, these results could still be compared within the study.

The venous Haemoglobin (Hb) content increased during MED infusion, while more stable values were detected when MK was added to the treatment (II). Another study has reported increased packed cell volume values (PCV) with high doses of DMED infusion (Pascoe et al. 2006), but those investigators failed to report Hb values. In a more recent investigation, arterial Hb as well as PCV, increased during DMED CRI (Pascoe 2015). It may be suggested that peripheral vascular effects could, at least in part, explain these findings, as vasoconstriction during MED administration will contract the vascular volume and decrease peripheral blood flow. The quantity of red blood cells is unlikely to change, and this could result in a relative increase in Hb levels during vasoconstriction, despite concurrent fluid administration. Further proof toward this postulate, is the fact that MK-467 reversed the effect on Hb (II). Others have postulated a potential reason for the Hb/PCV increase to be the contraction of vascular volume associated with diuretic effect or an alpha-2 mediated release of red blood cells from the spleen (Pascoe 2015).

Our results failed to reveal changes in plasma glucose levels (I, II) similar to other CRI studies (Lin et al. 2008). Administration of MED has been previously reported to produce a slow (peak in 2-4 hours post-administration) and non-significant change in plasma glucose concentrations (Burton et al. 1997; Ambrisko & Hikasa 2002), although one study has described an earlier and transient decrease in glucose level in dogs (Raekallio et al. 2005). If there were a peak in glucose levels in our studies, our sampling periods may have been too short to reveal them.

6.3.2. Sedation

In the study on conscious dogs with IM administration of both MED and MK-467, no differences between treatments were detected in the maximal sedation scores and all dogs were clinically sedated (III). Our results are consistent with previous reports where addition of MK-467 failed to alter the clinical sedation in dogs (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012). However, we demonstrated a faster onset and shorter duration of sedation with all of the doses of MK-467, consistent with a recent report that used intravenous administration of 1:25 ratio of MED:MK-467 that revealed shortened sedation (Bennett et al. 2016b). Our dose ratios were 1:10, 1:20, and 1:30, covering both sides of the dose ratio used in the aforementioned report. No difference was detected in the MK-467 dose used for the degree of its effect, although the duration of MED sedation had a trend to be reduced in a dose-dependent fashion.

Because the main observer was aware of the given treatments, the subjective composite sedation scores could have been inadvertently affected causing error on the results. However, these sedation scores were only a secondary variable to be investigated. In addition, the results are similar to previous data from "blinded" studies (Honkavaara et al. 2008; Rolfe et al. 2012; Bennett et al. 2016b). Moreover, these subjective findings were supported by the parallel changes in objective measurements of plasma concentrations.

6.4. Clinical implications

The overall results suggest that low doses of MED CRI may be used during inhalation anaesthesia in dogs in order to minimise the hemodynamic consequences. The questions remaining are whether these low doses of MED CRI could induce clinically efficacious analgesia, muscle relaxation, sedation and anaesthetic sparing. The MED-MK infusions administered in combination revealed that a certain balance of MED:MK ratio should be maintained in order to ascertain minimal cardiovascular consequences in either direction (agonist-antagonist) of the adrenergic effect spectrum. Further PK-PD investigations would potentially specify the optimal ratio. The simultaneous intramuscular administration of these drugs revealed challenges in interpreting absorption kinetics of MED altered by MK-467, which also influenced the haemodynamic effects. Although no

significant dose-dependency of the degree of the effects of IM MK-467 could be demonstrated with the MK-467 doses studied, the duration of these effects of MED appeared to be shortened in a dose-dependent fashion.

7. Conclusions

1) The haemodynamic responses of MED when given as IV infusions were dose-dependent during isoflurane anaesthesia (I, II). Increasing the plasma concentration of MK-467 decreased the cardiovascular effects of MED during IV infusions (II). However, there was a lack of dose-proportionality for MK-467 when administered at three different doses IM mixed in the same injection with MED (III).

2) The cardiovascular responses of MED followed the plasma concentrations of the drug with the doses and dose rates used (I-III). In addition, MK-467 initially increased, and thereafter decreased, the DMED plasma concentrations, which paralleled with the clinical effects seen (III).

3) There appears to be a complicated pharmacological interaction between MED and MK-467 that is dependent on the route and rate of administration, the injected concentration ratios, and achieved plasma concentrations (II-III). For MED, the absorption, distribution and elimination kinetics are simultaneously affected by MK-467, which in turn affect the plasma concentrations achieved and thus also the pharmacodynamic effects produced. Therefore, the recommendations for dose rates and dose ratios remain to be established and are dependent on the context.

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