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Haemodynamic Effects of MK-467 in combination with Medetomidine and Selected Sedative and Anaesthetic Agents in Dogs

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ACADEMIC DISSERTATION

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To my Family

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ABSTRACT

Medetomidine, an α_2-adrenergic agonist, is widely used in veterinary practice as a sedative and premedication agent, as it provides reliable sedation, anxiolysis and muscle relaxation. However, the marked haemodynamic changes limit its use to only healthy patients. MK-467, a peripheral α_2 -adrenoceptor antagonist, has been demonstrated to attenuate or prevent the early haemodynamic disturbances induced by medetomidine with intravenous co-administration in dogs.

The main aim of this series of investigations was to evaluate the haemodynamic effects of MK-467 when it is combined with medetomidine and selected sedative or anaesthetic agents that are commonly used in veterinary practice in dogs. More specifically, the concomitant administration of MK-467, medetomidine and butorphanol via intravenous or intramuscular routes was studied and compared with sedation induced with medetomidine-butorphanol. The haemodynamic effects after the various doses of MK-467 in relation to the standard dose of medetomidine were evaluated prior to and during isoflurane anaesthesia. In addition, the haemodynamic effect of the administration of MK-467 as a constant rate infusion was assessed during medetomidine-alfaxalone total intravenous anaesthesia. As a comparison, the haemodynamic effects of the co-administration of MK-467 and medetomidine as a premedication regimen in relation to acepromazine-butorphanol or glycopyrrolate-medetomidine prior to and during isoflurane anaesthesia were assessed. Finally, the influence of MK-467 on plasma dexmedetomidine and alfaxalone concentrations was determined.

After intravenous administration of MK-467 and medetomidine with or without butorphanol heart rate, cardiac output and oxygen delivery were well maintained, and the increase in systemic vascular resistance and blood pressures was prevented. The profitable effects of MK-467 appeared more slowly after intramuscular than after intravenous administration. The initial medetomidine-induced haemodynamic effects were dose-dependently attenuated by MK-467 during the premedication period but not during isoflurane anaesthesia with these studied doses of MK-467 in relation to a standard dose of medetomidine as a premedication regimen. The addition of MK-467 to medetomidine and alfaxalone produced a stable haemodynamic outcome when they were administered as constant rate infusions. Cardiac output and oxygen delivery were well maintained during both inhalation and total intravenous anaesthetic regimens when MK-467 was involved. The haemodynamic effects after the co-administration of medetomidine-MK-467 or acepromazine-butorphanol mimicked each other prior to and during isoflurane anaesthesia. Pre-treatment with subcutaneous glycopyrrolate failed to improve the medetomidine-induced reduction in cardiac output by means of increasing heart rate prior to or during isoflurane anaesthesia. Peripheral α_2- adrenergic receptor blockade by MK-467 resulted in a reduction in plasma dexmedetomidine and alfaxalone concentrations, presumably by altering the haemodynamic function.

In conclusion, the addition of MK-467 in a medetomidine-contained sedation or anaesthetic regimen attenuates the rather dramatic medetomidine-induced haemodynamic changes by blocking peripheral α_2-adrenergic receptors and by decreasing the plasma concentrations of dexmedetomidine. The overall haemodynamic stability is well maintained during either an isoflurane anaesthesia or an alfaxalone constant rate infusion. The combination of MK-467 with medetomidine may offer an alternative premedication regimen to the combination of acepromazine and butorphanol in healthy dogs.

LIST OF PUBLICATIONS

This thesis is based on the following original publications:

I Salla K, Restitutti F, Vainionpää M, Junnila J, Honkavaara J, Kuusela E, Raekallio M and Vaino O. The cardiopulmonary effects of a peripheral alpha-2-adrenoceptor antagonist, MK-467, in dogs sedated with a combination of medetomidine and butorphanol. *Veterinary Anaesthesia and Analgesia*, 2014, 41, pp 567-574.

II Salla K, Bennett RC, Restitutti F, Junnila J, Raekallio M and Vainio O. A comparison in dogs of medetomidine, with or without MK-467, and the combination of acepromazine-butorphanol as premedication prior to anaesthesia induced by propofol and maintained with isoflurane. *Veterinary Anaesthesia and Analgesia*, 2014, 41, pp 163-173.

III Bennett RC, Salla K, Raekallio MR, Scheinin M, Vainio O. Effects of α_2-adrenoceptor agonist medetomidine on the distribution and clearance of alfaxalone during coadministration by constant rate infusion n dogs. *American Journal of Veterinary Research*, 2017, 78, pp 956-964.

IV Salla KM, Tuns CI, Bennett RC, Raekallio MR, Scheinin M, Kuusela E, Vainio OM. Cardiovascular effects of premedication with medetomidine alone and in combination with MK-467 or glycopyrrolate in dogs subsequently anesthetized with isoflurane. *American Journal of Veterinary Research,* 2017, 78, pp 1245-1254.

The publications are referred to in the text by their roman numerals (I - IV).

ABBREVIATIONS

aBP	Arterial blood pressure
AUC_{last}	Area under the time-concentration curve to the last sampling point
AV	Atrioventricular
BIS	Bispectral Index
CaO_2	Arterial oxygen content
CI	Cardiac index
CNS	Central nervous system
CO	Cardiac output
CRI	Constant rate infusion
CSS	Composite sedation score
CVP	Central venous pressure
DAP	Diastolic arterial pressure
DMED	Dexmedetomidine
DO_2I	Oxygen delivery index
EMG	Electromyography
GABA	γ-aminobutyric acid
Hb_a	Arterial haemoglobin concentration
HR	Heart rate
IM	Intramuscular
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
LiDCO	Lithium dilution cardiac output
MAP	Mean arterial pressure
MED	Medetomidine
NA	Noradrenaline
PaO_2	Arterial partial pressure of oxygen
$PaCO_2$	Arterial partial pressure of carbon dioxide
$P_{(A-a)}O_2$	Alveolar to arterial difference in partial pressure of oxygen
RR	Respiratory rate
SAP	Systolic arterial pressure
SC	Subcutaneous
SD	Standard deviation
SAP	Systolic arterial pressure
SV	Stroke volume
SVI	Stroke volume index
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance index
TIVA	Total intravenous anaesthesia
VPC	Ventricular premature contraction

1 INTRODUCTION

Sedation is commonly warranted in small animal clinical practise to ensure safe patient handling and to perform minor procedures or diagnostic imaging. The appropriate premedication as a part of balanced anaesthesia facilitates the smooth induction and maintenance of anaesthesia. The ideal sedative or premedication regimen would provide reliable sedation, anxiolysis, muscle relaxation and analgesia, while the influence on the haemodynamic and pulmonary function would be minimal. In addition, it would be reversible.

Medetomidine (MED), a selective α_2-adrenoceptor agonist, provides reliable sedation, anxiolysis and muscle relaxation (Murrell & Hellebrekers 2005). Other beneficial effects of MED include its analgesic properties and the reduced requirement of other anaesthetic agents. The disadvantage of α_2- adrenergic agonists is the profound haemodynamic side effects, which are limiting their use to only healthy patients (Murrell & Hellebrekers 2005). These effects are mainly a consequence of the activation of peripheral α_2-receptors in the vasculature, leading to vasoconstriction, followed by hypertension (Pypendop & Verstegen 1998). This early hypertensive phase initiates a marked decrease in heart rate, cardiac output and oxygen delivery to tissues (Pypendop & Verstegen 1998). These peripherally mediated haemodynamic consequences of α_2-adrenoceptor agonists can be prevented or attenuated with the administration of MK-467 (Enouri et al. 2008a; Honkavaara et al. 2011), a peripheral alpha$_2$-adrenoceptror antagonist (Clineschmidt et al. 1988), while preferred centrally mediated effects are maintained (Restitutti et al. 2012; Bennet et al. 2016).

This thesis aimed to assess whether the beneficial haemodynamic effects after the co-administration of MED and MK-467 are sustained during the administration of selected sedative and anaesthetic agents that are commonly used for canine sedation and anaesthesia in clinical practice. In addition, a comparison to various premedication regimens was made to evaluate whether the co-administration of MED and MK-467 could provide any advantages concerning haemodynamic soundness. The following literature review focuses on the α-adrenergic regulation of the cardiovascular system and briefly summarises the pharmacodynamic effects of MED and selected sedative or anaesthetic agents. Later on, certain pharmacological agents used for modifying MED-induced haemodynamic effects are introduced.

2 REVIEW OF THE LITERATURE

2.1 Alpha-adrenergic receptors

Alpha-adrenergic receptors belong to the family of cell membrane G-protein-linked receptors (Gilman 1987), and they are widely distributed within central nervous system (CNS) and peripheral tissues (Gyires et al. 2009). Their expression and activity are highly tissue- and species-specific. Alpha-adrenergic receptors are activated by endogenous catecholamines, such as noradrenaline (NA) and adrenaline, which are the main mediators of the sympathetic nervous system. However, the physiological result of the activation of α-adrenergic receptors depends on the effector organ, location, the type and the subtype of the receptors, and the intracellular signalling mechanism (Docherty 2010; Gyires et al. 2009). Alpha-adrenergic receptors are divided into two types, α_1-and α_2-receptors (Langer 1974). Based on pharmacological radio ligand binding and functional studies and, later on, on the use of molecular cloning techniques and transgenic animal models, both receptor types are further divided into three subtypes (Docherty 1998). For α_1-adrenoceptors, these subtypes are α_{1A}-, α_{1B}- and α_{1D}-adrenoceptors (Docherty 2010), while α_2-adrenoceptors are divided into α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors (Bylund 1985; Calzada & de Artinano 2001). These receptors can be located junctionally or extrajunctionally, depending on whether or not the receptors are innervated by the noradrenergic nerves, respectively (Fig. 1) (Ruffolo 1985; Long & Kirby 2008). Junctional receptors may be located pre- or postsynaptically, while extra-junctional receptors are always postsynaptical by their nature (Long & Kirby 2008).

Junctional presynaptic α_2-adrenergic receptors are located in noradrenergic neurons in the CNS and peripheral sympathetic neuroeffector junctions. Based on an *in vitro* study using tissue preparations obtained from either wild type or transgenic mice, all three α_2-adrenergic subtypes participate in the negative feedback regulation of NA release in postganglionic sympathetic neurons (Trendelenburg et al. 2003), whereas in the CNS, the main subtypes regulating the NA release are the α_{2A}- and α_{2C}-adrenoceptor subtypes (Altman et al. 1999; Hein et al. 1999; Trendelenburg et *al.* 2001). The activation of these presynaptic receptors elicits signal transduction cascades, resulting in the inhibition of adenylate cyclase activity, the activation of potassium channels and the inhibition of voltage-gated calcium channels (Hayashi & Maze 1993; Piascik et al. 1996). This, in turn, leads to the inhibition of NA release from nerve endings (Langer 1974). Ascending and descending noradrenergic pathways originating in the nucleus of the brainstem in the CNS, the *Locus Coeruleus (LC),* regulate sleep and wakefulness, nociception as well as autonomic and endocrine responses (Scheinin & Schwinn 1992). The α_{2A}-adrenoceptors are expressed densely in the *LC* (Correa-Sales et al. 1992; Scheinin et al. 1994; MacDonald & Scheinin 1995) and have been demonstrated to be the subtype responsible for the sedative effects of α_2-agonist drugs, such as dexmedetomidine, which did not induce sedation in a α_{2A}-knock-out mice (Hunter et al. 1997). This subtype also regulates sympathetic outflow, as the sympathetic activity and circulating NA concentration are increased and followed by persisting resting tachycardia in α_{2A}-knock-out mice (Altman et al. 1999; Hein et al. 1999: Makaritsis et al. 1999). Antinociceptive effects, the impairment of thermoregulation (Hunter et al. 1997) and anaesthetic-sparing effects (Lakhlani et al. 1997) are also proposed to be mediated by α_{2A}-adrenoceptors.

Postsynaptical α_2-receptors, predominantly α_{2A}- and α_{2B}-receptors (Blaxall et al. 1994), mediate various functions in peripheral effector organs or tissues, such as the kidneys, liver, pancreas, uterus, adipose tissue and platelets (Sinclair 2003; Gyires et al. 2009). However, the following discussion focuses mainly on the cardiovascular effects of α_1- and α_2-adrenergic receptors.

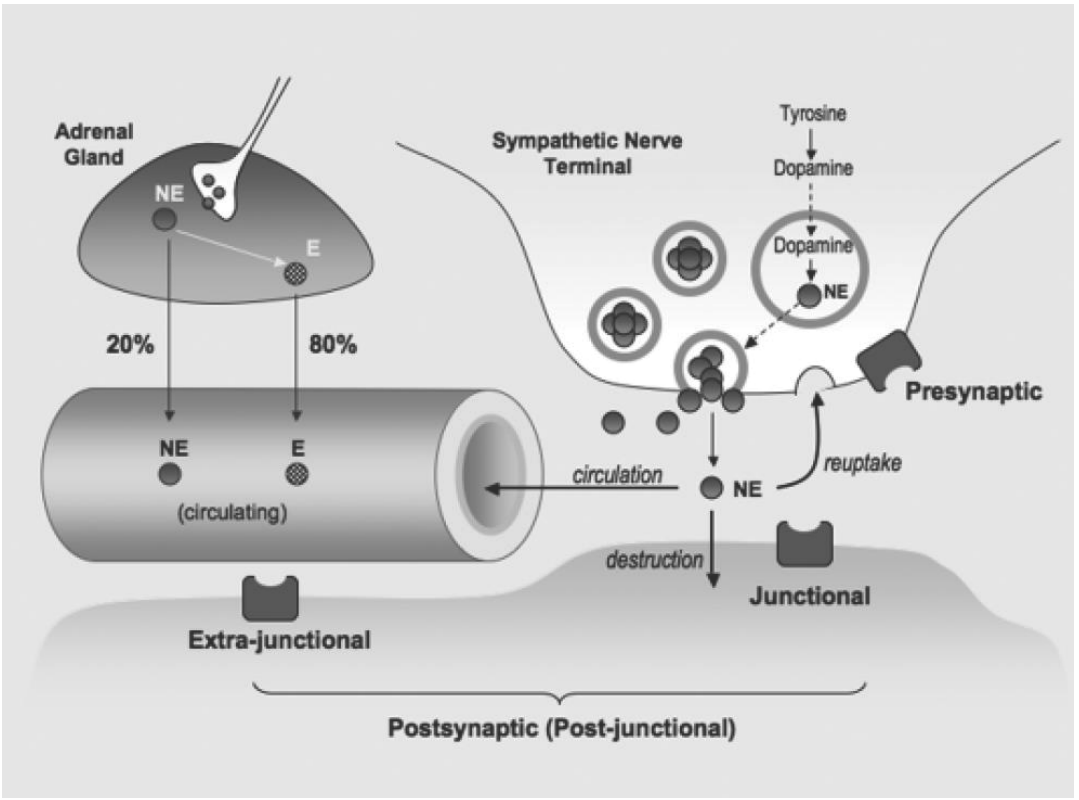

Figure 1. *Locations of adrenergic receptors in sympathetic nerve terminal and effector organs. Noradrenaline (NE) synthesized within the sympathetic nerve terminal interacts primarily with junctional and presynaptic adrenoceptors. Adrenaline (E) and NE released from the adrenal gland into the circulation, stimulates mainly extra-junctional adrenoceptors (Long & Kirby 2008).*

2.2 Cardiovascular regulation via alpha-adrenergic receptors

2.2.1 Regulation of vascular tone

Blood vessels are densely innervated by sympathetic neurons (Tsuru et al. 2002). Under normal conditions, the basal tone of the sympathetic nervous system maintains partial vascular smooth muscle contraction, distributing blood to the metabolic needs of the tissues, which, in turn, is strictly controlled by local control mechanisms of blood flow (Tsuru et al. 2002; Long & Kirby, 2008; Clifford 2011). Both α_1- and α_2-adrenergic receptors play the most important role in the regulation of vasomotor tone (Piascik et al. 1996). These receptors are responsible mainly for the vasopressor responses in both the arterial and the venous vasculature (Appleton et al. 1986;

Leech & Faber 1996), although differences between vascular beds exist (Horn et al. 1982; Kwan 1999).

The postsynaptic receptors mediating the basal vasomotor tone are mainly α_1-adrenergic receptors, which are activated by catecholamines released from either sympathetic nerve endings or adrenal medulla (Langer et al. 1981; Ruffolo 1985; Piascik et al. 1990; Vargas & Gorman 1995; Docherty 2010). Although vasoconstrictive action mediated by α_1-adrenergic receptors has been demonstrated in several *in vitro* studies using arterial or venous tissue preparations from various animal species (Flavahan et al. 1987; Argyle & McGraft 2000; Guimaraes & Moura 2001; Docherty 2010), the contribution to each α_1-adrenoceptor subtype on the regulation of the vascular smooth muscle tone has not been established (Guimaraes & Moura 2001; Docherty 2010).

The extra-junctional postsynaptic α_2-adrenergic receptors (Fig.1), which are located in the vascular smooth muscles, elicit vasoconstriction via the translocation of extracellular calcium (Piascik et al. 1996). These receptors are not directly activated by the sympathetic nerve fibres, but rather by circulating endogenous or exogenous ligands (Langer et al. 1981). Based on the transgenic mice models, the postsynaptic α_{2B}-adrenoceptors are the main subtypes responsible for arterial vasoconstriction (Link et al. 1996; Makaritsis et al. 1999). On the other hand, α_{2A} –adrenoceptor subtype predominates in the venous vasculature (Paiva et al. 1999; Guimaraes & Moura 2001). Additionally, α_{2A}- and α_{2C}-adrenoceptor subtypes are also involved in the vasoconstriction at distal arteries (Guimaraes & Moura 2001).

The decrease in sympathetic outflow from the CNS due to the activation of α_{2A}-adrenoceptors in the brainstem will lead to centrally mediated hypotension (MacMillan et al. 1996). This hypotensive effect is also lost in α_{2A}-deficient mice (Altman et al. 1999). As mentioned earlier, blood vessels are densely innervated by sympathetic nerve fibres, which may release various neurotransmitters, such as NA, adenosine 5'-triphosphate (APT) and neuropeptide Y (Chiba et al. 2003). Therefore, the activation of the prejunctional α_2-adrenoceptors leads to the inhibition of NA release and augmentation of the central hypotensive effect (Guimaraes & Moura 2001). Furthermore, vascular endothelial cells also express α_{2A}-adrenergic receptors (Shafaroudi et al. 2005), but in contrast to receptors located in vascular smooth muscle, the activation of these endothelial receptors leads to vasodilatation via the production of endothelium-derived nitrous oxide (Snapir et al. 2009; Wong et al. 2010). Endothelial α_{2A}-adrenergic receptors are activated with low concentrations of dexmedetomidine, while a higher concentration of dexmedetomidine elicits contraction in *ex vivo* preparations from the aortic and mesenteric artery rings of rats (Wong et al. 2010). The endothelium-dependent relaxation effect of dexmedetomidine is more pronounced in smaller arteries than larger ones (Wong et al. 2010).

2.2.2 Regulation of cardiac function

In general, cardiac function is influenced by interactions of the sympathetic and parasympathetic nervous systems. The magnitude of the cardiac response to the neural stimuli is dependent on the present activity of both divisions of the autonomic nervous system, while the response is not a direct summation of them (Levy 1984). As the sympathetic nervous system has a role in regulating the function of the heart, various types of adrenergic receptors are distributed in the myocardium. By using pharmacological radio ligand binding studies with functional imaging techniques, the main adrenergic receptor types within the myocardium have been found

to be β_1-receptors, but also α_1-adrenergic receptors are present in a minor density (Brodde & Michel 1999). In general, the activation of β_1- and α_1-adrenergic receptors in the myocardium leads to positive chronotropic- and inotropic effects. Although systemic administration of a selective α_2-adrenoceptror agonist has major effects on the cardiac function, to the author's knowledge, postjunctional α_2-receptors are lacking in the myocardium and sinoatrial node, as α_2-adrenoceptror agonist promote no direct myocardial depressant effects in isolated heart preparations (Housmans 1990; Flacke et al. 1992; Hongo et al. 2016). However, the activation of α_2-adrenoceptrors may indirectly decrease the cardiac function via different mechanisms. Firstly, a prominent activation of vascular postjunctional α_2-adrenoceptors in the vascular smooth muscle initiates vasoconstriction, which leads to an increase in systemic vascular resistance and afterload. In autonomically denervated dogs – i.e. the heart rate is maintained stable – this marked increase in the afterload decreases the cardiac index (CI) despite the simultaneous marked elevation in cardiac filling pressure (Flacke et al. 1990; Schmeling et al. 1991). In autonomically intact anaesthetised dogs, the decrease in CI is further accompanied by marked bradycardia due to the activation of the baroreceptor reflex arch evoked by hypertension (Bloor et al. 1992; Flacke et al. 1993). Secondly, the vasoconstriction of coronary arteries is mediated by an α_2- adrenoceptor agonists (Flacke et al. 1993). Therefore, an excessive activation of these receptors may decrease the oxygen supply to the myocardium, which may lead to myocardial dysfunction (Flacke et al. 1993). Nevertheless, Roekaerts et al. (1996) did not detect changes in the myocardial oxygen extraction ratio although the myocardial oxygen delivery was reduced, the oxygen demand was also diminished after the administration of an α_2- adrenoceptor agonist. Thirdly, the activation of junctional presynaptic α_{2A}-adrenergic receptors in the CNS decreases the activity of the sympathetic nervous system and thus indirectly suppresses cardiac function (Kubo & Misu 1981; Bloor et al. 1992; Villamil-Hernandez et al. 2013). In addition, the activation of the presynaptic α_2-receptors in sympathetic neuroeffector sites or in sympathetic ganglions (McCallum et al. 1998) may inhibit the neurotransmitter release from the sympathetic nerves innervating the myocardium and further modulate sympathetic transmission. A recent *in vitro* study demonstrated that a selective α_2-adrenoceptor agonist inhibited the increase in left ventricular pressure induced by sympathetic stimulation in isolated guinea pig hearts (Hongo et al. 2016).

2.3 Pharmacodynamic effects of certain sedative and anaesthetic drugs

2.3.1 Medetomidine

Medetomidine (MED) (IUPAC 5-[1-(2,3-di methylphenyl)ethyl]-1H-imidazole) is a highly α_2-adrenergic selective agonist, with an α_2:α_1 ratio of 1620:1 (Virtanen et al. 1988). MED produces consistent sedation, anxiolysis and muscle relaxation, which makes it a popular sedative agent in veterinary medicine. It is a racemic mixture of two enantiomers, dexmedetomidine (DMED) and levomedetomidine. The first is considered to be responsible for the clinical effects of MED (MacDonald et al. 1991; Savola & Virtanen 1991), while levomedetomidine is considered to be a clinically non-active enantiomer (Doze et al. 1989; MacDonald et al. 1991; Schmelling et al. 1991; Kuusela et al. 2001a). Medetomidine has no preferential affinities for different α_2-adrenoceptor

subtypes (Schwartz & Clark 1998), but DMED has been demonstrated to be a full agonist of α_{2B}-adrenoceptors and to have only partial selectivity for α_{2A}- and α_{2C}-adrenoceptors (Peltonen et al. 1998). The sedative and hypnotic effects of DMED are mediated via activation of central junctional presynaptic α_{2A}-adrenergic receptors located in the *LC* (Doze et al. 1989; Hunter et al. 1997; Scheinin & Schwinn 1992), while the analgesic effects are mediated via α_{2A}- and α_{2C}-adrenergic receptors located in the dorsal horn of the spinal cord (Sabbe et al. 1994; Stone et al. 1997; Fairbanks et al. 2002).

The haemodynamic effects of MED and DMED have been well described in the literature. In brief, they induce excessive vasoconstriction by stimulating the postjunctional α_2-adrenergic receptors located in the vascular smooth muscle, as discussed in a previous section. The resulting hypertension causes reflex bradycardia and arrhythmias, such as atrioventricular (AV) -blocks. Subsequently, CI and oxygen delivery (DO_2) are extensively reduced (Bloor et al. 1992; Pypendop & Verstegen 1998). The initial phase is followed by central sympatholysis and a subsequent inhibition of NA release, which may further promote the sustained poor haemodynamic function (Ebert et al. 2000). This biphasic blood pressure effect has also been described in dogs (Schmelling et al. 1991; Pypendop & Verstegen 1998). Nevertheless, true hypotension after MED is rarely reported in conscious dogs. These MED- and DMED-induced haemodynamic changes are also accompanied by reduced blood flow in various organs, such as the spleen (Lawrence et al. 1996; Restitutti et al. 2013; Rossi et al. 2016), kidneys (Lawrence et al. 1996; Restitutti et al. 2013) and skin (Lawrence et al 1996).

Both MED and DMED reduce the respiratory rate (RR) (Pypendop & Verstegen 1998; Kuusela et al. 2001a; Lerche & Muir 2004). In addition, after the systemic administration of MED or DMED, a response to the increased carbon dioxide tension is decreased in conscious (Sabbe et al. 1994) and anaesthetised dogs (Lerche & Muir 2006). However, they induce rather minimal effects on arterial oxygen or carbon dioxide partial pressures when administered alone to conscious dogs (Sabbe et al. 1994; Pypendop & Verstegen 1998). On the other hand, a concomitant administration of MED and opioids/benzodiazepines may elicit a significant respiratory depression accompanied by hypoxemia and hypercapnia (Pypendop et al. 1996; Raekallio et al. 2009). These respiratory effects of MED are reversed by the administration of atipamezole, an α_2-adrenergic antagonist (Vainio 1990).

Anaesthetic sparing effects after MED administration have been described (Bufalari et al. 1996; Bloor et al. 1992; Kuusela et al. 2001b; Pascoe et al. 2006; Maddern et al. 2010; Pinelas et al. 2014; Pascoe 2015). In dogs undergoing surgery, DMED or MED constant rate infusions (CRI) have been used as an adjunct in balanced anaesthesia to provide analgesia and to reduce the need for anaesthetic agents used for maintenance, such as isoflurane (Uilenreef et al. 2008; Rioja et al. 2013). In more detail, in experimental settings, the infusion of DMED 1 μg/kg/h in healthy dogs anaesthetised with isoflurane (ET_{ISO} 1.3%) has been shown to suppress the nociceptive withdrawal reflex and temporal summation (Lervik et al. 2012), and an 18% decrease in isoflurane minimum alveolar concentration (MAC) has been detected already with a very low DMED CRI (0.5 μg/kg/h) (Pascoe et al. 2006). Typical MED-induced haemodynamic changes in isoflurane-anaesthetised dogs are dose-dependent, with the doses ranging from 0.2 to 12 μg/kg/h (Kaartinen et al. 2010). A DMED or MED CRI has also been used in conscious dogs for prolonged sedation (Grimm et al. 2005; Lin et al. 2008; Carter et al. 2010; Lamont et al. 2012), where dose-dependent sedation has been induced with infusion rates varying between 1 and 2

μg/kg/h (Lamont et al. 2012), but significant haemodynamic changes were already observed after 1 μg/kg/h (Carter et al. 2010).

2.3.2 Butorphanol

Butorphanol (BUT) (IUPAC 17-cyclobutylmethyl-3,14-dihydroxymorphinan) is a synthetic opioid with mixed agonist-antagonist properties and an affinity for μ-, κ- and δ- opioid receptor subtypes (Commiskey et al. 2005). BUT is well absorbed after intramuscular and subcutaneous administration, and its sedative action is reported to be mild in dogs (Pfeffer et al. 1980). However, a ceiling effect on the sedative and anaesthetic sparing effects, as well as visceral antinociceptive properties, have been suggested after the administration of increasing doses of BUT (Murphy et al. 1982; Houghton et al. 1991; Sawyer et al. 1991).

In conscious dogs, a high dose of BUT (0.2-0.8 mg/kg) causes a mild to moderate but significant decrease in the HR, MAP, CI and arterial partial pressure of oxygen (PaO_2) (Sederberg et al. 1981; Trim 1983; Sawyer et al. 1991). However, no changes in HR were recorded after the IM or IV administration of BUT (0.1 mg/kg) in conscious dogs (Ambrisko et al. 2005; Girard et al. 2010). In dogs anaesthetised with an inhalant anaesthetic agent, BUT (0.2-0.4 mg/kg) decreased HR and arterial blood pressures (aBP) (Tyner et al. 1989; dos Santos et al. 2011). The decrease in CO was detected after the administration of BUT in isoflurane- (Tyner et al. 1989), but not in desflurane-anaesthetised dogs (dos Santos et al. 2011). The decrease in HR after BUT could be due to an inhibition of glycinergic neurotransmission in cardiac vagal nerve fibres via the activation of κ- receptors in the nucleus ambiguous (Wang et al. 2004). Despite these mild haemodynamic effects, BUT (0.2 mg/kg) did not affect the spleen perfusion evaluated by contrast-enhanced ultrasound (Rossi et al. 2016). Ventilatory depression has been demonstrated after the administration of BUT in either conscious or anaesthetised dogs (Dodam et al. 2004; dos Santos et al. 2011), but the extent of hypoventilation is smaller than with pure μ-agonist opioid (Horan & Ho 1989; Dodam et al. 2004).

Butorphanol is frequently combined with an α_2- adrenergic agonist, such as MED, to enhance the level and quality of sedation and analgesia in dogs (Ko et al. 1996; Ko et al. 2000a; Kuo & Keegan 2004; Girard et al. 2010). In more detail, the synergistic effect on sedation was also reported after IV administration of low doses of MED (1 μg/kg) and BUT (0.1 mg/kg), as the combination produced significantly deeper sedation in dogs than either agent alone (Girard et al. 2010). Synergistic or additive effects on nociception have been demonstrated in rats after the administration of an opioid and MED (Ossipov et al. 1990). In addition, the induction dose of thiopental was lower with MED-BUT premedication than with MED alone (Muir et al. 1999). BUT also reduces the incidence of nausea and vomiting after the administration of an α_2-agonist, especially in cats (Papastefanou et al. 2015). The haemodynamic effects after the co-administration of MED and BUT are likely due to MED, as even low doses of MED (1 μg/kg) and BUT (0.1 mg/kg) resulted in a similar decrease in HR as MED alone, while no difference in HR was detected with this dose of BUT in comparison to placebo (Girard et al. 2010). Furthermore, comparable changes in haemodynamic effects have been described after the administration of MED-BUT with midazolam followed by partial reversal after the administration of atipamezole (Pypendop et al. 1996). In addition, BUT (0.4 mg/kg) and DMED (5 μg/kg) administered concomitantly IM resulted in a significant and sustained decrease in CO and valvular regurgitation to the heart as assessed by echocardiography in dogs (Kellihan et al.

2015). Despite the significant haemodynamic changes after the combination of an α_2-agonist and BUT, myocardial cell damage based on the serum concentrations of the cardiac muscle biomarker cardiac troponin I has not been detected in healthy dogs (Singletary et al. 2010). On the contrary, the BUT-induced changes in neuroendocrine and metabolic function, such as increased plasma concentrations of adrenaline and cortisol, are blunted with concomitant administration of MED (Ambrisko et al. 2005).

2.3.3 Acepromazine

Acepromazine (ACP) (IUPAC (10-[3-dimethylamino) propyl] phenothiazine-2yl methyl ketone)) is a phenothiazine derivate that produces sedation and tranquilization via the blocking of central dopamine (D_2) receptors (Nybäck & Sedvall 1968). However, when used for premedication, ACP is often combined with opioid analgesics. Even though ACP may reduce the reaction to the external stimuli, it is generally considered to lack of antinociceptive properties (Barnhart et al. 2000; Wegner et al. 2008; Bergadano et al. 2009).

ACP has been demonstrated to decrease arterial blood pressure with an associated increase in heart rate when administered alone in conscious (Popovic et al. 1972, Turner et al. 1974; Monteiro et al. 2008) or isoflurane-anaesthetised dogs (Monteiro et al. 2007). A decrease in cardiac function with an accompanied decrease in systemic arterial blood pressures has been reported in conscious dogs with ACP combined with opioids (Stepien et al. 1995). The decrease in blood pressure is suggested to be due to the blocking of peripheral α_1-adrenoceptors, as ACP has been demonstrated to diminish the effects of phenylephrine, a known α_1-adrenoceptor agonist, on MAP (Ludders et al. 1983). Studies demonstrating the α_1-adrenoceptors' antagonistic effects have also been conducted with another phenothiazine derivate, such as chlorpromazine (Foster et al. 1954), whose chemical structure is similar to that of ACP. While the main tranquilization effects of ACP are mediated via the blocking of central dopaminergic actions, a peripheral blockade of dopaminergic receptors may also influence the cardiovascular function (Frishman & Hotchkiss 1996). Monteiro et al. (2007) demonstrated that previous administration of ACP blunted the vasopressor effects of dopamine. Although ACP may decrease arterial blood pressure, the following refractory increase in HR via the baroreceptor reflex arch results in well-maintained cardiac output (Monteiro et al. 2007). Other beneficial effects of ACP are due to its antiarrhytmogenic properties. ACP increases the arrhythmogenic dose of adrenaline in dogs anaesthetised with halothane (Dyson & Pettifer 1997). Thus, ACP might provide protective properties against cardiac arrhythmias. Respiratory rate may be reduced after the administration of ACP, but ACP has little effect on minute ventilation based on the blood gas analysis (Popovic et al. 1972; Turner et al. 1974).

Despite its well-reported vasodilation effects, ACP did not prevent bradycardia nor hypertension induced by DMED (Alvaides et al. 2008). However, the hypertension lasted a shorter period than with DMED alone (Alvaides et al. 2008). Furthermore, the concomitant administration of ACP and an α_2-adrenoceptor agonist did not prevent the incidence of conduction abnormalities, e.g. first- or second-degree AV blocks, in dogs (Sarchahi et al. 2009; Saponaro et al. 2013).

2.3.4 Propofol

Propofol (IUPAC 2,6-diisopropylphenol) is a short-acting lipid-soluble intravenous anaesthetic agent, that is commonly used for the induction or maintenance of anaesthesia. Its anaesthetic properties are mediated via γ-aminobutyric acid ($GABA_A$) -receptors in CNS (Ying et al. 2005). Propofol has been demonstrated to cause a decrease in SVR and, subsequently, in MAP (Goodchild & Serrao 1989; Brüssel et al. 1989). Moreover, a direct negative inotropic action in chronically instrumented dogs has also been demonstrated by Pagel & Warltier (1993). Propofol induction is often accompanied by hypoventilation or apnoea, and the extent of hypoventilation depends on the dose and speed of injection (Keates & Whittem 2012; Amengual et al. 2013).

Medetomidine premedication reduces the induction dose of propofol (Bufalari et al. 1996; Kojima et al. 2002; Sano et al.2003; Ko et al. 2006), but the ventilatory parameters do not differ from those of propofol alone (Bufalari et al. 1996). Propofol induction followed by a CRI has been demonstrated to induce a mild but significant increase in HR and a decrease in MAP in MED premedicated dogs (Vainio 1991). Intramuscular premedication with MED (10 μg/kg) has been reported to influence on the pharmacodynamics of propofol, i.e. lower plasma propofol concentrations were needed to maintain general anaesthesia in dogs (Hall et al. 1994). Additionally, the administration of MED significantly reduced the apparent volume of distribution of propofol, while systemic clearance was not affected (Hall et al. 1994). More recently, propofol administered as target controlled infusion resulted in lower plasma propofol concentrations with DMED than with ACP (Bell et al. 2011).

2.3.5 Alfaxalone

Alfaxalone (ALF) (IUPAC 3α-hydroxy-5α-pregnane-11,20-dione) is a neuroactive steroid whose anaesthetic action is mediated by the enhancement of $GABA_A$-mediated inhibitory synaptic transmission (Weir et al. 2004). No differences between the sexes have been detected in pharmacokinetic parameters of ALF in dogs (Ferre et al. 2006). It has no effect on CO nor left ventricular contractility when used alone with a labelled anaesthetic dose (2mg/kg IV), but it induces a decrease in SVR and aBP, followed by tachycardia, with supraclinical doses (6 and 20 mg/kg IV) (Muir et al. 2008). In addition, when ALF (2 mg/kg IV) was combined with BUT and midazolam, no significant changes were detected in the function of the left ventricle, as assessed by echocardiography (Seo et al. 2015). A dose-dependent decrease in RR, minute volume and PaO_2 as well as an increase in $PaCO_2$ have been reported after ALF (Muir et al. 2008; Keates & Whittem 2012).

Furthermore, it is possible to induce anaesthesia with IM administration of ALF combined with MED and BUT in healthy dogs (Lee et al. 2015). However, significant reductions in CO, stroke volume (SV) and left ventricular contractility as assessed by echocardiography were detected from 10 minutes after treatment until the recovery (Lee et al. 2015). In addition, this combination has also been shown to decrease RR and increase the alveolar to arterial difference in partial pressure of oxygen ($P_{(A-a)}O_2$) (Lee et al. 2015).

A constant rate infusion of ALF has been used successfully to maintain general anaesthesia during surgical procedures in dogs (Suarez et al. 2012; Herbert et al. 2013; Warne et al. 2015; Conde Ruiz et al. 2016). The cardiopulmonary effects of an ALF CRI have been evaluated when it is infused alone in non-premedicated (Quiros Carmona et al. 2014) or premedicated dogs

(Ambros et al. 2008). In both studies, an ALF CRI induced mild clinically acceptable changes in haemodynamic function, where HR and CI were maintained, but a mild decrease in aBP were detected (Ambros et al. 2008; Quiros Carmona et al. 2014). Concomitant CRIs of ALF and DMED lead to expected dose-dependent changes in haemodynamic function induced by an α_2-agonist (Quiros Carmona et al. 2014).

2.3.6 Ketamine

Ketamine (IUPAC 2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1-one) is a racemic mixture of two enantiomers, S- and R- ketamine (White et al. 1982). Ketamine is a dissociative anaesthetic agent, and its action is mediated via several mechanisms, including an interaction with N-methyl-D-aspartate (NMDA) glutamate, opioid, muscarinic and monoaminergic receptors as well as voltage-gated Ca^{2+} channels (Hirota & Lambert, 1996; Quibell et al. 2015). However, the main anaesthetic and analgesic function is mediated via the blocking of NMDA glutamate receptors in the CNS (Anis et al. 1983). Ketamine-induced anaesthesia with high dose of ketamine (10 mg/kg), when administered as a sole anaesthetic, is accompanied with sustained laryngeal reflexes, salivation and muscle rigidity (Haskins et al. 1985; Ambrisko et al. 2005). Thus, the administration of ketamine for sedation or induction purposes, is recommended to be combined with agents that provide good muscle relaxation, such as α_2- agonists or benzodiazepines, to facilitate and smoothen the sedation or induction.

In addition to the anaesthetic and analgesic properties, ketamine may modify cardiopulmonary function. Increases in HR, aBP and CO have been reported after the administration of a high dose of ketamine (10 mg/kg) administered either intramuscularly (Ambrisko et al. 2005) or intravenously (Traber et al. 1968; Haskins et al. 1985) in dogs. This dose of ketamine was also accompanied with an increase in the plasma concentrations of noradrenaline and cortisol (Ambrisko et al. 2005). Moreover, IV administration of ketamine (10 mg/kg) has been demonstrated to increase HR, aBP and DO_2 in the haemorrhagic hypovolemia model in dogs (Haskins & Patz 1990). The stimulatory responses of the cardiovascular system to ketamine, have been demonstrated to be related to the CNS effects of ketamine (Ivankovich et al. 1974). These responses are proposed to be elicited by the stimulation of sympathetic outflow from the CNS, as these effects were attenuated in sympathetically blocked dogs (Traber & Wilson 1969; Pagel et al. 1992) or in Starling lung-heart preparations (Traber et al. 1968). Therefore, certain sedative or anaesthetic agents that reduce sympathetic outflow from the CNS may blunt this response (Ivankovich et al. 1974; Levänen et al. 1995). However, a transient increase in HR and aBP has been detected after the administration of ketamine in dogs premedicated with MED (Hellebrekers & Sap 1997; Enouri et al. 2008a; Ko et al. 2001a; Ko et al. 2013).

2.3.7 Midazolam

Midazolam (IUPAC 8-chloro-6-(2-fluorophenyl)-1-methyl-4H-imidazo[1,5-a][1,4] benzodiazepine) is a benzodiazepine derivate, and it is commonly used as an adjunctive drug in veterinary practice to induce central muscle relaxation or anticonvulsant effects. It is commonly combined with anaesthetic induction agents, such as ketamine (Hellyer et al. 1991), propofol (Stegmann & Blester 2001) or alfaxalone (Seo et al. 2015) to reduce the amount of induction agent and to facilitate smooth intubation.

The mechanism of action of benzodiazepines is mediated by enhancing the endogenous GABA affinity to the $GABA_A$-receptors in the CNS, resulting in the hyperpolarization of postsynaptic cell membranes (Rudolph et al. 1999). The sedative effects of midazolam are unpredictable in dogs; paradoxical excitement has been described after the administration of midazolam despite the co-administration of acepromazine or methadone in healthy dogs (Simon et al. 2014). Moreover, the co-administration of midazolam with dexmedetomidine did not enhance the sedation in comparison to dexmedetomidine alone, but the combination reduced the induction dose of propofol in healthy dogs (Canfran et al. 2016). In dogs, a midazolam CRI causes a modest dose-dependent reduction in the MAC of enflurane (Hall et al. 1988), and the MAC_{NM} (i.e. end-tidal concentration of isoflurane that prevents movements) of isoflurane (Seddighi et al. 2011). Additionally, diazepam has been demonstrated to potentiate a fentanyl-induced decrease in isoflurane MAC in dogs (Hellyer et al. 2001). However, a ceiling effect appears with higher doses (Hall et al. 1988; Seddighi et al. 2011). The cardiovascular effects induced by bentzodiazepines, either midazolam or diazepam, are considered to be minor in dogs (Jones et al. 1979). After IV administration of a high dose of midazolam (1-10 mg/kg), the MAP decreased, but HR and CO were well maintained (Jones et al. 1979; Gelman et al. 1983). Furthermore, midazolam has no effect on adrenaline-induced arrhythmogenesis in dogs anaesthetised with halothane (Court et al. 1993).

2.3.8 Isoflurane

Isoflurane (IUPAC 2-chloro-2-(difluoromethoxy)-1,1,1-trifluoroethane) is a methyl ethyl ether and is halogenated with five fluorines and one chlorine, and it was discovered in 1965 (Eger 1981). Today, it is one of the most commonly used inhaled anaesthetic agents in veterinary medicine.

Isoflurane suppresses haemodynamic and ventilatory function dose-dependently (Steffey & Howland 1977; Eger 1981; Brahim & Thut 1984; Bernard et al. 1990; Mutoh et al. 1997). Isoflurane has a direct effect on cardiac function, leading to decreased contractility (Pagel et al. 1991a). In dogs, isoflurane is a very potent vasodilator in comparison to other inhalation agents, such as desflurane, enflurane and halothane (Pagel et al. 1991b). In autonomically intact dogs, vasodilation and the subsequent decrease in aBP cause the inhibition of the baroreceptor firing, leading to an increase in HR and a reduction in SV while CO is well maintained in clinically relevant isoflurane end-tidal concentrations (i.e. 1.25 times MAC) (Pagel et al. 1991b). Moreover, studies on chronically instrumented dogs have demonstrated that, despite the decrease in resistances in a various vascular beds, the systemic, coronary, cerebral, hepatic and renal blood flows are well maintained (Bernard et al. 1990; Bernard et al. 1991; Merlin et al. 1991; Pagel et al. 1991a,b). The mechanism of the vasodilatory effect of isoflurane is suggested to be due to the activation of adenosine-triphosphate-sensitive potassium channels in vascular smooth muscle (Kersten et al. 1998; Cason et al. 1994; Iida et al. 1998). In addition, halogenated inhalant agents, such as isoflurane, have been demonstrated to decrease the endothelin-1-mediated vasoconstrictive effect in rat aortas (Boillot et al. 1995). $Alpha_2$-adrenoceptor agonists and isoflurane have an opposing effect on the vasomotor tone, as isoflurane acts as a functional antagonist to the α-adrenergic mediated vasoconstriction in dogs (Kenny et al. 1989; Kersten et al. 1993) However, to the author's knowledge, the exact mechanism of the inhibition of α-adrenoceptors by isoflurane has not been fully established.

The minimum alveolar concentration (MAC) of isoflurane in dogs has been reported to be approximately 1.28% (Steffey & Howland 1977). Various sedative, anaesthetic and analgesic agents reduce the MAC of isoflurane. More specifically, acepromazine alone (Heard et al. 1986) or combined with an opioid (Monteiro et al. 2016) reduces the inhalant anaesthetic agent's MAC by 30% - 60 %, while α_2-adrenoceptor agonists, reduce the MAC of isoflurane by up to 88% (Bloor et al. 1992; Ewing et al. 1993; Lerche & Muir 2006; Pascoe et al. 2006; Pascoe 2015).

2.4 Certain drugs that modify medetomidine-induced haemodynamic responses

2.4.1 Atipamezole

Sedation and analgesia induced with an α_2-agonist can be reversed with a centrally acting α_2-adrenoceptor antagonist, such as atipamezole (IUPAC 4-(2-ethyl-1,3-dihydroinden-2-yl)-1H-imidazole) (Clarke & England 1989; Virtanen et al. 1989; Vainio 1990; Vainio & Vähä-Vahe 1990; Vähä-Vahe 1990). Based on receptor binding studies, atipamezole is a highly α_2-adrenoceptor-selective antagonist, as its affinity to α_2-adrenergic receptors is 8,526 times higher than to α_1-adrenergic receptors (Virtanen et al. 1989). In addition to central α_2-adrenergic receptor blockade, atipamezole also blocks peripheral α_2-adrenergic receptors (Virtanen et al. 1989). Therefore, atipamezole is also able to reverse MED-induced bradycardia (Vähä-Vahe 1990). However, transient hypotension presumably due to vasodilatation might be accompanied with the administration of atipamezole for the reversal of medetomidine-induced sedation (Vainio 1990).

Furthermore, atipamezole also modifies the elimination kinetics of MED, as the IM administration of atipamezole reduces the area under time- concentration curve (AUC_{last}) of MED in MED–sedated dogs (Salonen et al. 1995). Although, both atipamezole and MED are metabolized by hydroxylation in the liver, no metabolic interaction has been detected (Salonen et al. 1995).

2.4.2 MK-467

MK-467, also known as L-659,066, (IUPAC N-[2-[2R, 12bS]-2'-oxospiro[1,3,4,6,7,12b-hexahydro-[1]benzofuro[2,3-a]quinolizine-2,5'-imidazoline]-1'-yl]ethyl]methane sulphonamide) is an α_2-adrenoceptror antagonist, which has greater selectivity for α_2- than α_1-adrenergic receptors, as in *in vivo* and *in vitro* studies the α_2:α_1 ratios were 30:1 and 105:1, respectively (Clineschmidt, et al. 1988). Its pharmacodynamic effects have been demonstrated to be mediated mainly peripherally, as the brain/plasma ratio for MK-467 was 0.06 and 0.04 in rats and primates, respectively (Clineschmidt et al. 1988). Additionally, MK-467 did not reverse clonidine-induced centrally mediated mydriasis (Clineschmidt et al. 1988), or the sedative effects of DMED in rats (Doze et al. 1989). Moreover, DMED's antiarrhytmogenic action on epinephrine-induced arrhythmias in halothane-anaesthetized dogs was blocked with atipamezole but not with MK-467 (Hayashi et al. 1991). Later on, several studies regarding the haemodynamic, sedative, analgesic or pharmacokinetic effects of MK-467 with various α_2-adrenoceptor agonists in several species have been published (Table 1).

In dogs, IV administration of MK-467 (250 μg/kg) alone did not cause any clinically relevant adverse effects (Honkavaara et al. 2011). A transient increase in HR, CI and DO_2 and a slight decrease in SVR were detected after the administration of MK-467, while the MAP was maintained (Honkavaara et al. 2011). Similar findings were reported by Enouri et al. (2008b) shortly after the IV administration of MK-467 (200 μg/kg). This compensatory response in HR and CI has been proposed to be sympathetically mediated, secondary to a reduction in peripheral

Table 1. *Published studies on the pharmacological effects of MK-467 in certain animal species and in humans.*

Species	α_2-adrenergic agonist	Administration Route of MK-467/ Outcome	Reference
Dog	DMED	IV / Arrythmogenicity of DMED	Hayashi et al. 1991
	DMED	IV / Haemodynamics	Pagel et al. 1998
	DMED	IV / Bradycardia and Sedation	Honkavaara et al. 2008
	DMED	IV / Dose-dependency, Haemodynamics	Honkavaara et al. 2011
	DMED	IV / BIS and Clinical Sedation	Restitutti et al. 2011
	DMED	IV / Pharmacokinetics	Honkavaara et al. 2012
	DMED	IV /Metabolic changes	Restitutti et al. 2012
	DMED	IV / Organ Blood Flow	Restitutti et al. 2013
	DMED	CRI / MAC of Sevoflurane	Hector et al. 2017
	MED	IV /Haemodynamics	Enouri et al. 2008b
	MED	IV and IM /Haemodnamics	Rolfe et al. 2012
	MED	IV and IM / Thermography	Vainionpää et al. 2013a
	MED	CRI /Dose-dependency, Haemodynamics	Kaartinen et al. 2014
	MED	IV / Sedation and Analgesia	Bennet et al. 2016
	MED	IM / Pharmacokinetics, Haemodynamics	Restitutti et al. 2017
Cat	DMED	IV / Pharmacokinetics	Pypendop et al. 2016
	DMED	IV /Bradycardia and Sedation	Honkavaara et al. 2017a
	DMED	IV / Haemodynamics	Pypendop et al. 2017a
	DMED	IM / Sedation, HR and Blood pressures	Honkavaara et al. 2017b
	DMED	IM / Pharmacokinetics	Pypendop et al. 2017b
Horse	MED	IV / HR and Blood pressures	Bryant et al. 1998
	Detomidine	IV / Sedation, HR, CVP, Pharmacokinetics	Vainionpää et al. 2013b
	Detomidine	IV / Haemodynamics	Pakkanen et al. 2015
	Romifidine	IV / Pharmacokinetics, HR, Blood pressures	de Vries et al. 2016
Sheep	MED	IV / HR and Blood pressures	Bryant et al. 1998
	DMED	IV / Haemodynamics	Raekallio et al. 2010
Rat	Clonidine	Various *in vitro* and *in vivo* studies	Clineschmidt et al. 1988
	NA	IV / Haemodynamics	Szemeredi et al. 1989
Human	Clonidine	PO / Metabolic changes, HR, Blood pressures	Warren et al. 1991
	NA	IV / Metabolic changes	Schafers et al. 1992
	NA	IV / Metabolic changes, HR, Blood pressures	Sciberras et al. 1994

NA, not administered

vascular tone as a result of α_2-adrenergic receptor blockade (Szemeredi et al. 1989; Pagel et al. 1998). Cardiac arrhythmias or significant respiratory effects has not been observed in dogs after IV administration of MK-467 (Honkavaara et al. 2011).

MK-467 is able to attenuate the early haemodynamic effects of DMED and MED in conscious dogs when administered via the IV route by using either a pre-emptive administration of MK-467 (Pagel et al. 1998; Enouri et al. 2008b) or a concomitant bolus administration with DMED (Honkavaara et al. 2008; Honkavaara et al. 2011). MK-467 has also been demonstrated to prevent DMED-CRI-induced haemodynamic effects in halothane-anaesthetised dogs, leading to lower aBP and higher HR than with a DMED-CRI alone (Hayashi et al. 1991). In more detail, the administration of MK-467 has been found to prevent increases in SVR and aBP induced by α_2-adrenoceptor agonists, followed by a significantly higher HR, CI and DO_2 with the concomitant administration of MK-467 and an α_2-adrenoceptor agonist than with an α_2-adrenoceptor agonist alone (Enouri et al. 2008b; Honkavaara et al. 2011). Based on the overall haemodynamic stability during a 90-minute observation period, the optimal dose ratio for sedation has been suggested as 1:50 for the concomitant IV administration of DMED (10 μg/kg) and MK-467 (500 μg/kg), respectively (Honkavaara et al. 2011). However, in isoflurane–anaesthetised dogs, a clinically acceptable haemodynamic outcome was achieved with constant rate infusions of MED and MK-467, when the MED:MK-467 ratio was between 1:18 and 1:50 (Kaartinen et al. 2014). After an IM administration of MK-467 and MED in dogs, an initial transient decrease in HR and CO, and increases in SVRI and aBP, were recorded irrespective of the doses of MK-467 (Rolfe et al. 2012; Restitutti et al. 2017).

The overall respiratory effects of MK-467 in dogs sedated with α_2 –adrenergic agonists could be considered to be minor (Enouri et al. 2008b; Honkavaara et al. 2011). In more detail, the decrease in RR or fall in PaO_2 induced with 10 μg/kg of DMED in dogs was not influenced by the presence of any studied dose of MK-467 (250, 500 or 750 μg/kg IV) (Honkavaara et al. 2011). However, RR tended to be slightly higher in dogs sedated with MK-467 and DMED than with DMED alone (Honkavaara et al.2011). Furthermore, the two highest doses of MK-467, i.e. 500 and 750 μg/kg, combined with DMED (10 μg/kg IV) caused a statistically significant increase in arterial partial pressure of carbon dioxide ($PaCO_2$), but this finding was not clinically relevant as none of the values exceed 45 mmHg (Honkavaara et al. 2011).

In dogs, the concomitant administration of MK-467 with DMED or MED did not influence the level of sedation assessed by composite sedation scoring method (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012). More recently, the duration of sedation and the latency for leg withdrawal time have been reported to be shorter in dogs sedated with MED and MK-467 than with MED alone (Bennett et al. 2016). The presence of MK-467 has been shown to diminish the plasma concentrations of DMED in conscious dogs sedated with either DMED (Honkavaara et al. 2012) or MED (Bennett et al. 2016). These changes in plasma DMED concentrations were most likely influencing on the duration of sedation (Bennett et al. 2016). In more detail, the AUC_{last} of DMED was significantly smaller after concomitant IV administration of MK-467 (250 μg/kg) and DMED (10 μg/kg) than with DMED alone in conscious dogs (Honkavaara et al. 2012). However, higher doses, i.e. 500 or 750 μg/kg, of MK-467 had no further effect on the exposure of the DMED (Honkavaara et al. 2012). In contrast, DMED did not significantly influence the plasma concentrations of MK-467 at the dose of 250 μg/kg (Honkavaara et al. 2012). Intramuscular administration of MK-467 in the same syringe with MED facilitated the

absorption of MED, as the time of the peak plasma DMED concentration was sooner when MED was combined with MK-467 than with MED alone (Restitutti et al. 2017). Additionally, the peak plasma DMED concentration was significantly higher with MK-467 than without it (Restitutti et al. 2017). In contrast, the absorption of MK-467 appeared to be slower than that of MED, based on the later occurrence of the peak plasma concentration (T_{max}) of MK-467 compared to DMED (Restitutti et al. 2017).

2.4.3 Anticholinergic agents

Anticholinergic agents, such as atropine or glycopyrrolate (IUPAC (1,1-dimethylpyrrolidin-1-ium-3-yl) 2-cyclopentyl-2-hydroxy-2-phenylacetate;bromide), mediate their effects via competitive antagonism of acethylcholine at the muscarinic receptors in the postganglionic terminals in the parasympasympathetic pathway (Levy 1984; Ali-Melkkilä et al. 1993). Glycopyrrolate acts peripherally, as it has been demonstrated to not penetrate blood-brain-barrier in dogs, while atropine also elicits central effects (Proakis & Harris 1978). Anticholinergic agents increase the HR by blocking the muscarinic receptor on the sinoatrial and AV nodes. The magnitude of the increase in HR is related to the dose of the anticholinergic agent (Ali-Melkkilä et al. 1993), but it may also be dependent on the concurrent sympathetic tonus (Levy 1984). Bradycardia induced by α_2-adrenoceptor agonists could be prevented or treated by an IM or IV administration of anticholinergic agents, such as atropine or glycopyrrolate (Vaino & Palmu 1989; Short 1991; Alibhai et al. 1996; Ko et al. 2001b; Sinclair et al. 2002; Sinclair et al. 2003; Alvaides et al. 2008; Congdon et al. 2011). However, the increase in HR has been demonstrated to be accompanied with arrhythmias, such as second-degree AV block, ventricular premature contractions (VPC), bigeminy and pulsus alternans. Additionally, more severe hypertension has been detected when an anticholinergic agent is combined with an α_2-adrenoceptor agonist than after an α_2-adrenoceptor agonist alone. In general, these haemodynamic disturbances were milder when the anticholinergic agent was administered before the α_2-adrenoceptor agonist than when they were administered concomitantly (Short 1991; Alibhai et al. 1996; Congdon et al. 2011). On the other hand, an improvement in CI has been reported in dogs sedated with romifidine and pre-treated with IM glycopyrrolate (Sinclair et al. 2002). Subcutaneous (SC) glycopyrrolate administered twenty minutes before IM administration of MED was accompanied with fewer adverse effects than MED alone, as briefly reported by Muir et al. (1999). Moreover, no differences were detected in HR or non-invasive blood pressures during isoflurane anaesthesia with or without glycopyrrolate in this study (Muir et al. 1999). Enouri et al. (2008b) demonstrated that the IV administration of glycopyrrolate together with MK-467 ten minutes prior to MED did not yield any advantage as regards haemodynamic function when compared with dogs treated with MK-467 alone prior to MED. To the author's knowledge, the haemodynamic effects of SC pre-treatment with an anticholinergic agent prior to an α_2-adrenoceptor agonist has not been widely studied and never compared with MK-467.

3 AIMS OF THE STUDY

The main objective of this thesis was to evaluate the haemodynamic effects of MK-467 when combined with medetomidine and selected sedative or anaesthetic agents that are commonly used in veterinary practice in dogs.

The more detailed aims were:

1. To investigate the haemodynamic effects of intramuscular and intravenous administration of MK-467 on dogs sedated with medetomidine and butorphanol. (I)

2. To explore the haemodynamic effects of various doses of MK-467 in relation to a standard dose of medetomidine prior to and during isoflurane anaesthesia. (II, IV)

3. To compare the co-administration of MK-467 and medetomidine as a premedication regimen with acepromazine-butorphanol or glycopyrrolate-medetomidine prior to and during isoflurane anaesthesia. (II, IV)

4. To assess the haemodynamic effects of MK-467 administered as a constant rate infusion during total intravenous anaesthesia with medetomidine and alfaxalone. (III)

5. To evaluate the influence of peripheral α_2-adrenergic receptor blockade by MK-467 on plasma dexmedetomidine and alfaxalone concentrations. (III, IV)

4 MATERIALS AND METHODS

4.1 Animals

The same eight laboratory purpose-bred beagles (six neutered males and two neutered females) were used in all studies I–IV. The dogs were 3–5 years old during the studies and weighted 13.7 ± 1.8 kg. The dogs were housed as a group and fed with commercial dry dog food twice a day. The dogs received vaccinations and deworming in a regular basis. They were considered healthy based on a clinical physical examination, complete blood count and serum chemistry. Before the experiments, food was withheld for 12 hours, but water was supplied *ad libitum* until the transfer to the research room. The dogs were familiarized with the research room and personnel before the studies. The studies were approved by the Animal Experiment Board of Finland (ESHL-2007-05199/Ym-23 and ESAVI-2010-07734/Ym-23).

4.2 Instrumentation

Before each treatment trial, the dogs were instrumented under isoflurane anaesthesia. Prior to anaesthesia, the cephalic vein was cannulated with a 20-gauge catheter (Terumo Europe V.N., Belgium) and propofol (Propovet 10 mg/mL; Abbott Laboratories Ltd, UK) was administered until the effect (maximum 6 mg/kg) to induce anaesthesia and to ensure orotracheal intubation. Lidocaine (maximum 1 mg/kg) was administered locally under the skin before the insertion of a 7 Fr double-lumen central venous catheter (CV-12702, Arrow International, PA, USA). Additionally, the metatarsal artery was cannulated with a 22-gauge catheter (Terumo Europe V.N. Belgium) and secured in place. In study II, the heads of the dogs were clipped, shaved and washed to the ensure maximal contact of the bispectral index (BIS) electrode. The dogs were allowed to recover after extubation for at least 1 hour to ensure normal locomotion, behaviour and temperature before baseline measurements. The dogs received acetated Ringer's solution at 10 mL/kg/hr IV during the instrumentation.

4.3 Study design and treatments

All studies were of a prospective, randomized cross-over design by nature, with an at least two-week washout period. The randomization was performed blindly by picking up a paper note from an envelope prior to the experiments. The author was not blinded to the treatments; as cardiopulmonary variables were recorded objectively by the numeric measurements from the monitors. However, whenever subjective assessments were made, the observer was blinded to the treatments (II, III). Treatments within studies I–IV are summarized in Table 2.

Medetomidine (Dorbene 1 mg/mL, Laboratories Syva S.A. Leon, Spain) and MK-467 (Merck, Sharpe & Dohme, Philadelphia, PA, USA) were used in all studies. MK-467 was supplied as a powder, and before each trial, MK-467 was dissolved into saline to a concentration of 10 mg/mL (I, II, III). The doses of MK-467 used in study IV were lower than in the other studies, and MK-467 was therefore dissolved into saline to a concentration of 2 mg/mL to ensure the correct dosage. Butorphanol (Butordol 10 mg/mL, Intervet International B.V., Netherlands) was used with MED or ACP (Plegicil 10 mg/mL, Pharmaxim, Sweden) in studies I and II, respectively. In study IV, glycopyrrolate (Robinul 0.2 mg/mL, Meda Pharma GmbH & Co., Bad

Homburg, Germany) was administered subcutaneously 15 minutes prior to IV premedication. All drugs were drawn up separately and mixed in a single syringe before the administration of premedication (I, II, IV) or loading dose (III). When necessary, saline was added to achieve an equal total volume in all treatments within all studies. All IV injections were administered via a cephalic catheter over 30 seconds and flushed with 0.5 mL/kg of saline (I, II and IV) or over 60 seconds (III) with immediately followed by a CRI. The intramuscular injection (I) was administered with a 25-gauge hypodermic needle (BD Microlance 3, Becton Dickinson) into the *M. quadriceps lateralis*. Proper intramuscular administration was confirmed by applying negative pressure before the injection was given.

Anaesthesia was induced at 10 minutes (III) or 20 minutes (II, IV) after IV premedication with propofol (Propovet 10 mg/mL, Abbott Laboratories Ltd. UK), alfaxalone (Alfaxan 10 mg/mL, Vetoquinol UK Ltd, Buckingham, UK) or ketamine (Ketaminol Vet 50 mg/mL, Intervet International B.V., Boxmeer, The Netherlands) and midazolam (Midazolam Hamelin 5 mg/mL, Hameln Pharma Plus GmbH, Hameln, Germany) in studies II, III and IV, respectively. In studies II and IV, anaesthesia was maintained with isoflurane (Isoflo, Orion Pharma Ltd, Turku, Finland) in 100% oxygen with a constant 1.2% isolurane end-tidal concentration (ET_{ISO}) delivered via a circle system (Anesco Inc. KY, USA) and an isoflurane vaporizer (Ohmeda Isotec 3, BOC Health Care, UK). In the study III, each CRI regimen was administered via separate infusion pumps (IVAC 7201 Gold Signature, Alaris Medical System Inc., CA, USA; Perfusor Secura FT, B. Braun, Melsungen, Germany), and infusions were diluted in saline so that the sum of infusion rates in each CRI regimen was 11 mL/kg/hour. The dogs were breathing spontaneously in all studies.

Table 2. *Treatments and administration routes in studies I–IV.*

Study	Treatment	Sedation / Premedication	Induction/ Maintenance
I	MEDBUT	Medetomidine 20 µg/kg + Butorphanol 0.1 mg/kg IM/IV	
	MEDBUT-MK	MB + MK-467 500 µg/kg IM/IV	
II	MED	Medetomidine 10 µg/kg IV	Propofol to effect/ Isoflurane ET_{ISO} 1.2%
	MMK250	Medetomidine 10 µg/kg + MK-467 250 µg/kg IV	as MED
	ACEBUT	Acepromazine 0.01 mg/kg + Butorphanol 0.3 mg/kg IV	as MED
III	ALF	Saline IV	Alfaxalone 2.4 mg/kg + CRI 3.6 mg/kg/h
	MEDALF	Medetomidine 4 µg/kg + CRI 4 µg/kg/h IV	as ALF
	MEDALF-MK	MA + MK-467 150 µg/kg + CRI 120 µg/kg/h IV	as ALF
IV	MED	Medetomidine 10 µg/kg IV	Ketamine + Midazolam/ Isoflurane ET_{ISO} 1.2%
	MMK50	MED + MK-467 50 µg/kg IV	as MED
	MMK100	MED + MK-467 100 µg/kg IV	as MED
	MMK150	MED + MK-467 150 µg/kg IV	as MED
	MGP	Glycopyrrolate 10 µg/kg SC 15 minutes prior to MED	as MED

The dose ratio in studies I and II between MED and MK-467 was derived from previous a study with dogs (Honkavaara et al. 2011). In study III, the aimed target constant plasma drug concentrations were 1 mg/L for alfaxalone, 3 ng/mL for MED and 300 ng/mL for MK-467. Loading doses and CRI rates were calculated by using reported pharmacokinetic values for alfaxalone (Ferre et al. 2006), MED (Kuusela et al. 2000), and MK-467 (Honkavaara et al. 2012).

In study I, intramuscular atipamezole 50 µg/kg (Antisedan 5 mg/mL, Orion Pharma Ltd, Finland) was administered after the last measurement to speed up full recovery. Additionally, subcutaneous meloxicam 0.2 mg/kg (Metacam 5 mg/mL, Boehringer Ingelheim Vetmedica, Ingelheim/Rhein, Germany) was administered in all studies after the last measurements to reduce the inflammatory reaction and to ameliorate any discomfort due to minor tissue trauma from cannula insertions. After the study, when normal locomotion and temperature were achieved, the dogs returned to their facilities and were housed in individual cages for the following night then returned to their pack the following morning.

4.4 Cardiopulmonary measurements (I, II, III, IV)

After the connection of all wires and lines, the dogs were positioned in lateral recumbence and allowed to adapt before baseline measurements. The baseline recordings and measurements were taken at least five minutes before the administration of the drugs. Continuous lead II electrocardiography, as well as systolic (SAP), diastolic (DAP) and mean (MAP) arterial pressure and central venous pressure (CVP) were monitored in all studies using a multichannel monitor (Datex-Ohmeda S/5 Anesthesia Monitor, GE Healthcare, Finland). Additionally, inspired fraction of oxygen, end tidal carbon dioxide (II, III and IV) and end tidal isoflurane (ET_{ISO}) (II, IV) concentrations were recorded continuously after the induction of anaesthesia. The gas analyser was calibrated before the studies with the calibration gas supplied by the manufacturer (Quick Cal Calibration Gas, GE Healthcare, Finland). The pressure transducers (Gabarith PMSET, Becton Dickinson, UT, USA) were reset to atmospheric pressure and calibrated with a transducer stimulator tester (Delta-Cal Utah Medical Products Inc., UT, USA). The level of manubrium was used as a zero reference. Cardiac output measurements, arterial blood gas samples and rectal temperature were monitored at certain times points during the trials. Cardiac output was measured with the lithium indicator dilution method (LiDCO Plus Hemodynamic Monitor, LiDCO Ltd, UK) within all studies as described by Mason et al. (2001) using standard dose of 0.075 mmoL LiCl. The standard values of 10 g/L for haemoglobin and 140 mmoL/L for sodium were used and later corrected with actual values measured from simultaneously drawn arterial blood gas samples. Arterial blood gas samples were drawn anaerobically into pre-heparinized syringes (Pico-50, Radiometer, Copenhagen, Denmark) via the catheter, stored in iced water for no longer than 15 minutes and analysed (ABL 855, Radiometer, Copenhagen, Denmark). Temperature-corrected oxygen and carbon dioxide partial pressures (PaO_2 and $PaCO_2$, respectively), pH and bicarbonate (HCO_3^-) as well as arterial lactate, haemoglobin (Hb_a) and sodium concentrations were recorded. Haemoglobin oxygen saturation was calculated (Reeves et al. 1982). Subsequently, the cardiac index (CI), systemic vascular resistance index (SVRI), arterial oxygen content (CaO_2), tissue oxygen delivery index (DO_2I) and $P_{(A-a)}O_2$ (I) were calculated by using standard equations (Haskins et al. 2005). During sedation (I) and before the induction of anaesthesia (II, III and IV), the RR was measured by counting the chest movements

for one minute, and later on by means of capnography (II, III, IV). In study III, where somatic nociceptive stimulus was applied to the ventral surface of the tail using a nociceptive pressure tester (Selitto Randell Paw Pressure Tester, IITC Life Science Inc., Woodland Hills, CA, USA), the cardiopulmonary measurements were always taken before this stimulus.

4.5 Plasma concentrations of drugs (III, IV)

Blood samples for the analysis of plasma drug concentrations were taken at selected time points. Samples were taken from the central venous catheter to EDTA tubes and centrifuged at 3000G for 15 minutes. Until analysis, the separated plasma was stored at -20C. The plasma concentrations of both enantiomers of MED, as well as MK-467 and alfaxalone were analysed with a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method. More detailed descriptions of the analyses of plasma drugs concentrations are found in the original papers (III, IV). The AUC_{last} for both enantiomers of medetomidine, MK-467 and alfaxalone were calculated using a trapezoidal method with WinNonLin software.

4.6 Level of sedation, BIS and the quality of induction, intubation and recovery (II)

The level of hypnosis during the premedication and anaesthetic period was monitored by using BIS. BIS electrodes (BIS sensor; Aspect Medical Systems, MA, USA) were placed on clipped and disinfected skin in a frontal-temporal position as described by Campagnol et al. (2007). Additionally, the level of sedation was evaluated subjectively 5, 10 and 15 minutes after premedication by an experienced, blinded observer. A composite sedation score modified from Kuusela et al. (2001b) was used, whereby 0 was normal and 16 was deeply sedated. The quality of induction and intubation on a scale of 1 (smooth) – and 4 (rough/failed) (Maddern et al. 2010), and the quality of recovery on a scale of 1 (smooth) – 5 (extreme excitement), modified from Lozano et al. (2009), were subjectively assessed by the same blinded observer. In addition, extubation, righting, sternal and walking times in minutes were recorded. The induction agent doses needed for induction were also recorded in studies II and IV.

4.7 Statistical analyses

Data is presented as the mean ± standard deviation (SD) or median (minimum–maximum range) for parametric or ordinal data, respectively.

In each study, cardiopulmonary variables, CSS (II) and BIS (II) were analysed separately by using the statistical software SAS for Windows, version 9.2 (SAS Institute Inc., NC, USA). The change from the baseline measurement was calculated for all of the response variables, and the change was used as a response in the modelling. The differences between treatments in the change from baseline values were assessed with repeated measures analysis of covariance (RM ANCOVA) models. The model consisted of a baseline covariate, the main effects of treatment, the period (the order of treatments) and time point of measurement as well as two-way interactions of period*time point and treatment*time point as fixed effects, and the main effect of dog and two-way interaction terms of period * dog and time point * dog as random effects. The estimates of treatment effects were calculated both over time and by time point from the fitted models. For

the overall and time-specific treatment differences, 95% confidence intervals and p-values were calculated.

In study II, with continuous variables – e.g. time of extubation, righting, sternal and walking – measured only once, differences between the treatments were evaluated with analysis of covariance (ANCOVA) models. For extubation, righting and sternal times a logarithmic transformation was made to normalize the distribution. Here, the model included the main effects of treatment and period, and their two-way-interaction of treatment*period as fixed effects, and the main effect of dog, and the two-way-interaction of dog*period as random effects. For the variable walking time, the model was the same without the two-way interaction term treatment*period, because it had to be removed to enhance the model fit. As regards the three category variables, the quality of induction, intubation and recovery, only descriptive analysis was generated.

For pharmacokinetic data (III, IV), the normality of distributions was evaluated with Shapiro Wilk's test. Comparison between treatments for the AUC_{last} of dexmedetomidine, levomedetomidine and alfaxalone was analysed with SPSS statistical software using analysis of variance (ANOVA) and paired t-test. Bonferroni corrections were used when appropriate.

For all statistical analyses, a p-value of <0.05 was considered as statistically significant.

5 RESULTS

5.1 Haemodynamic effects

Data on HR, MAP, CI and SVRI from all studies are presented in Figures 2–5, respectively. The rest of the cardiopulmonary data is presented in Tables 3–6 from studies I–IV, respectively.

In general, MK-467 prevented the increase in systemic vascular resistance in all studies (Fig. 5). This was accompanied with better-maintained HR (Fig. 2), CI (Fig. 4) and DO_2I (Tables 3–6).

In more detail, MK-467 attenuated the increase in both MAP (Fig.3) and SVRI (Fig. 5) in dogs either sedated with MEDBUT (I) or anaesthetised with MEDALF-CRI (III). However, MAP (Fig. 3) remained significantly higher during the whole observation period in dogs premedicated with MED and anaesthetised with isoflurane (II, IV) than after the treatments which included any studied dose of MK-467. However, during isoflurane anaesthesia induced with either propofol (II) or ketamine and midazolam (IV), the SVRI with MED alone did not differ significantly from the SVRI with treatments including any studied dose of MK-467 (Fig. 5). Subsequently, HR (Fig. 2) remained significantly lower with MEDBUT (I), MED (II) and MEDALF (III) treatments than with treatments that included MK-467 during the whole observation period. In study IV, however, the decrease in HR was inversely related to the dose of MK-467 during the premedication phase. After IM administration of MK-467 together with MEDBUT (I), the reversing effects of MK-467 seemed to appear more slowly than after IV administration.

MK-467 attenuated the decrease in CI (Fig. 4) and DO_2I (Tables 3 and 5) when dogs were either sedated with MEDBUT (I) or anaesthetised with MEDALF-CRI (III). With a standard dose of intravenous MED, MK-467 induced dose-dependent effects on CI (Fig. 4) and DO_2I (Tables 4 and 6) during the premedication phase (II, IV). However, in comparison to MED, significantly higher CIs and DO_2Is were detected during isoflurane anaesthesia only shortly after the induction with propofol (II) with the highest studied dose of MK-467 (250 μg/kg) (MMK250), but not with lower doses of MK-467 after the induction with ketamine and midazolam (IV).

When comparing two different premedication regimens (II), ACEBUT versus MMK250, no significant differences were detected in haemodynamic function during the premedication phase between these two treatments, with the exception of MAP (Fig. 3), which was significantly lower with ACEBUT than with MMK250. During isoflurane anaesthesia induced with propofol (II), MAP (Fig. 3) remained lower with ACEBUT than with MMK250. Additionally, HR (Fig. 2) was higher during the entire anaesthetic phase with MMK250 than with ACEBUT, but CI (Fig. 4) and DO_2I (Table 4) were higher with MMK250 than with ACEBUT only shortly after the induction of anaesthesia.

In study IV, pre-treatment with subcutaneous glycopyrrolate 15 minutes prior to intravenous MED (MGP) augmented MED-induced changes in the SVRI (Fig. 5), aBP and CVP (Fig. 3 and Table 6), while MGP maintained a higher HR than MED during premedication phase (Fig. 2). However, CI (Fig. 4) and DO_2I (Table 6) were not proportionally improved by means of heart rate. Tachycardia (HR > 150 beats per minute; Haskins 2015) was not detected after MGP. During isoflurane anaesthesia, the CI (Fig. 4) was always lower with MGP than with MED or with MED combined with any studied dose of MK-467. Additionally, CI (Fig. 4) was significantly higher during the entire observation period with MMK150 than with MGP, with the exception of the 15-minute time point.

After MED bolus IV administration, bradyarrhythmias with second-degree AV-blocks were detected (I, II, IV). Intravenous co-administration of MK-467 (250 μg/kg) (MMK250) with medetomidine (10 μg/kg) (II) prevented second-degree AV blocks, but lower doses of MK-467 (IV) were not able to completely prevent them. Additionally, occasional single uniform VPCs with intermittent pulse deficits were noted with MGP (IV).

5.2 Respiratory effects and blood gases

Data on respiratory effects and arterial blood gases are presented in Tables 3–6 from studies I–IV, respectively.

In all studies, the RR decreased after each treatment (Tables 3–6), and further after the induction of anaesthesia with propofol (II) (Table 4), alfaxalone (III) (Table 5) or ketamine and midazolam (IV) (Table 6). However, differences in RRs were not detected between the treatments in the studies I, II and IV. During total intravenous anaesthesia (III), the RR was significantly higher with ALF than with both MEDALF and MEDALF-MK, but not between MEDALF and MEDALF-MK (Table 5). The administration route did not affect the RR in dogs sedated with MEDBUT with or without MK-467 (I) (Table 3). While the RR decreased in all studies after treatment, a subsequent increase in $PaCO_2$ was recorded. Co-administration of MK-467 with MEDBUT administered via either route (I) resulted in higher $PaCO_2$ over time than with MEDBUT alone (Table 3). A similar trend was detected after IV bolus administration of MED with the highest studied dose of MK-467 (MMK250) during the premedication phase (II) (Table 4), but not with the lower doses of MK-467 (IV) (Table 6). On the contrary, during isoflurane anaesthesia (II, IV), $PaCO_2$ was higher with MED than with MMK250 (II) (Table 4), or with MMK100 and MMK150 (IV) (Table 6). Similarly, during total intravenous anaesthesia (III), $PaCO_2$ was significantly lower with MEDALF-MK than with MEDALF, but higher than with ALF (III) (Table 5). In comparison to MED, premedication with ACEBUT (II) resulted in higher $PaCO_2$ during the whole observation period, but $PaCO_2$ was higher with ACEBUT than with MMK250 only during isoflurane anaesthesia (Table 4). However, $PaCO_2$ never exceed 60 mmHg in any study.

In addition to the changes in $PaCO_2$, a decrease in PaO_2 was detected after the treatments, and no differences were detected between the treatments prior to or during isoflurane anaesthesia (II, IV) (Tables 4 and 6). A transient but clinically significant decrease was detected in PaO_2 three minutes after MEDBUT-MK administration (I) via either route, but not with MEDBUT (Table 3). In addition, the initial $P_{(A-a)}O_2$ was significantly higher with MEDBUT-MK than with MEDBUT via either administration route, but $P_{(A-a)}O_2$ returned towards to the baseline value sooner with MEDBUT-MK than with MEDBUT (I) (Table 3). On the contrary, shortly after the induction with an alfaxalone loading dose (III), PaO_2 was significantly lower with MEDALF than with either ALF or MEDALF-MK (Table 5).

In all studies, the arterial lactate concentration remained lower than 2.5 mmol/L. However, minor changes in arterial lactate concentrations were detected in the studies I and II. It was significantly higher with MEDBUT than with MEDBUT-MK (I) and with MED than with both MMK250 and ACEBUT (Tables 3 and 4).

5.3 Plasma concentrations of drugs (III, IV)

The mean ± SD AUC_{last} for dexmedetomidine was significantly higher with MEDALF (195 ± 31 ng min/ml) than with MEDALF-MK (90 ± 15 ng min/ml) ($p < 0.05$) and with MED (155 ± 36 ng min/ml) than with MMK150 (101 ± 12 ng min/ml) ($p < 0.05$) in studies III and IV, respectively. Additionally, in study III, the AUC_{last} for alfaxalone was significantly higher with MEDALF (455,000 ± 85,000 ng min/ml) than with MEDALF-MK (239,000 ± 31,000 ng min/ml) ($p < 0.05$).

5.4 Other effects

5.4.1 The dose of induction agents (II, IV)

The mean ± SD propofol dose needed for induction (II) was 1.5 ± 0.3 mg/kg for MED, 2.1 ± 0.5 mg/kg for MMK250 and 2.3 ± 0.9 mg/kg for ACEBUT. Statistical difference was reached between each treatment ($p < 0.05$). In study IV, the doses for ketamine and midazolam needed for induction were 1 mg/kg and 0.2 mg/kg, respectively. In three exceptional cases, the ketamine dose was 1.5-2.0 mg/kg and midazolam 0.2-0.4 mg/kg.

5.4.2 Level of sedation and BIS (II)

The sedation scores (CSS) did not differ significantly between MED, ACEBUT and MMK250. Before the induction of anaesthesia, at the 15-minute time point, the CSS was 9 (7–14) for MED, 10 (6–11) for MMK250 and 10 (5–13) for ACEBUT when presented as median (range). Before the induction of anaesthesia, BIS recordings (mean ± SD) at the 10-minute time point were 80 ± 8 for MED, 83 ± 4 for MMK250 and 87 ± 6 for ACEBUT, and no significant differences were detected between the treatments at this time point (Table 4). During anaesthesia, all dogs were in the surgical plane of anaesthesia, based on a clinical evaluation of anaesthetic depth. However, the level of hypnosis according to BIS was significantly higher with MED than with both MMK250 and ACEBUT (Table 4). Significant differences were detected in EMG recordings between ACEBUT and MED or MMK250 during both the premedication phase and isoflurane anaesthesia (Table 4).

5.4.3 The quality of induction, intubation and recovery (II)

In general, the quality of induction, intubation and recovery was smooth with all treatments. However, some mild paddling or twitching was detected during induction in one dog after MED and two dogs after ACEBUT. Similarly, some mild paddling was detected in the recovery phase in one dog with MED and ACEBUT, and three dogs after MMK250.

The recovery phase was blindly assessed, and the quality of recovery was smooth in all treatments. However, there was a significant difference in recovery times between the treatments. The time in minutes (mean ± SD) from cessation of isoflurane to extubation and to walking was significantly shorter with MMK250 (7 ± 2 and 15 ± 5, respectively) than with MED (10 ± 3 and 33 ± 16, respectively) or ACEBUT (18 ± 10 and 39 ± 11, respectively).

5.4.4 Rectal temperature

Rectal temperature declined mildly over time in all studies, but no significant differences were detected between treatments in the studies III–IV (Tables 5 and 6). The rectal temperature was significantly lower in dogs sedated with MEDBUT-MK administered either IV or IM ($p < 0.001$ for both) (I), and after premedication with MMK250 ($p = 0.003$) or ACEBUT ($p = 0.04$) (II) than with MEDBUT and MED, respectively (Tables 3 and 4). Rectal temperature was significantly lower with MEDBUT-MK than MEDBUT from 20 to 30 minutes after drug administration (I), while significance was reached at all time points between MED and MMK250 (II). At the end of the observation period, the approximate difference in rectal temperatures was 1°C between MEDBUT-MK and MEDBUT (I), and 0.5°C between MED and MMK250 (II).

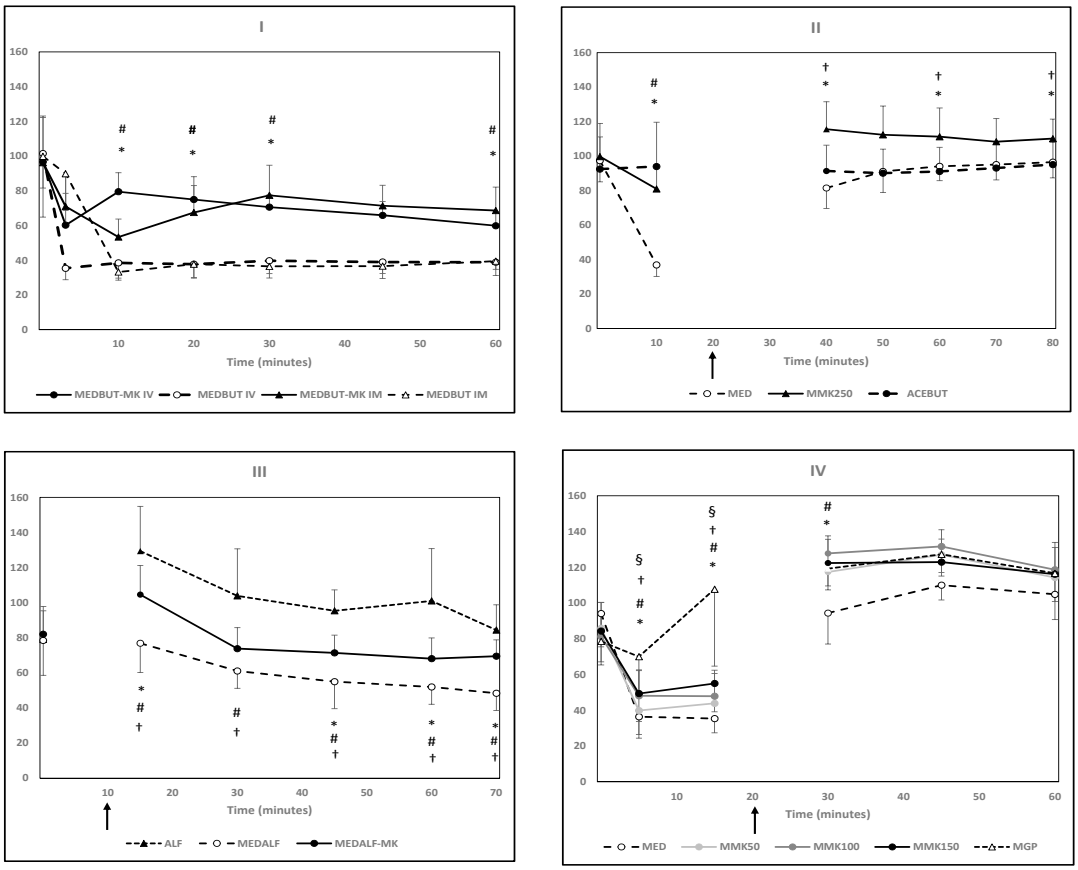

Figure 2. *Mean ± SD heart rates (beats/min) over time for eight dogs sedated with MEDBUT IV/IM, MEDBUT IV/IM (I); premedicated with MED (II, IV), MMK250 (II), ACEBUT (II), MGP (IV), MMK150 (IV) and MMK50 (IV) prior to isoflurane anaesthesia; or administered a CRI of ALF, MEDALF and MEDALF-MK (See Table 2 for treatment key). The black arrow indicates the time of the induction of anaesthesia with propofol (II), alfaxalone (III) or ketamine and midazolam (IV).* * *Significant difference between MEDBUT IV and MEDBUT-MK IV (I); MED and MMK250 (II) or MMK150 (IV); or MEDALF and MEDALF-MK (III) ($p < 0.05$). # Significant difference between MEDBUT-IM and MEDBUT-MK IM (I); MED and ACEBUT (II) or MGP (IV); or MEDALF and ALF (III) ($p < 0.05$). † Significant difference between MMK250 and ACEBUT (II); MEDALF-MK and ALF (III); or MMK150 and MGP (IV) ($p < 0.05$). § Significant difference between MMK150 and MMK50 (IV) ($p < 0.05$).*

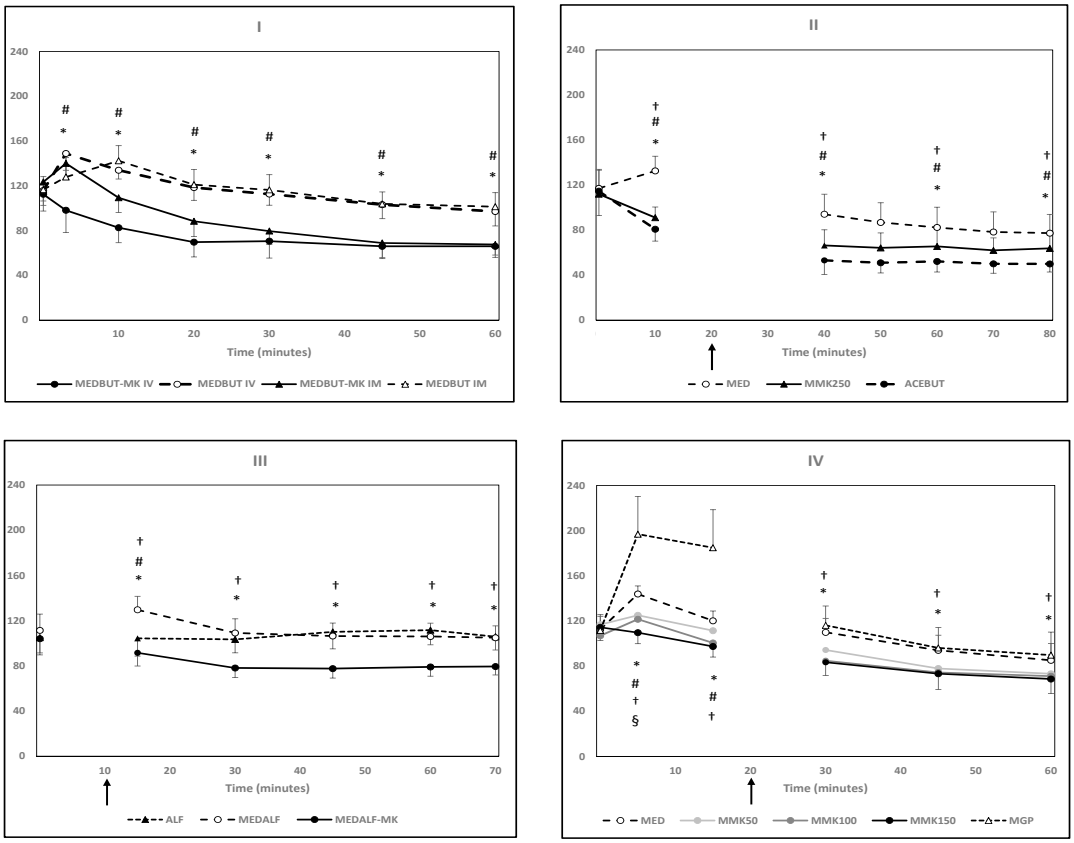

Figure 3. *Mean ±SD mean arterial pressures (mmHg) over time for eight dogs sedated with MEDBUT IV/IM, MEDBUT IV/IM (I); premedicated with MED (II, IV), MMK250 (II), ACEBUT (II), MGP (IV), MMK150 (IV) and MMK50 (IV) prior to isoflurane anaesthesia; or administered a CRI of ALF, MEDALF and MEDALF-MK (See Table 2 for treatment key). The black arrow indicates the time of the induction of anaesthesia with propofol (II), alfaxalone (III) or ketamine and midazolam (IV).*

** Significant difference between MEDBUT IV and MEDBUT-MK IV (I); MED and MMK250 (II) or MMK150 (IV); or MEDALF and MEDALF-MK (III) ($p < 0.05$). # Significant difference between MEDBUT-IM and MEDBUT-MK IM (I); MED and ACEBUT (II) or MGP (IV); or MEDALF and ALF (III) ($p < 0.05$). † Significant difference between MMK250 and ACEBUT (II); MEDALF-MK and ALF (III); or MMK150 and MGP (IV) ($p < 0.05$). § Significant difference between MMK150 and MMK50 (IV) ($p < 0.05$).*

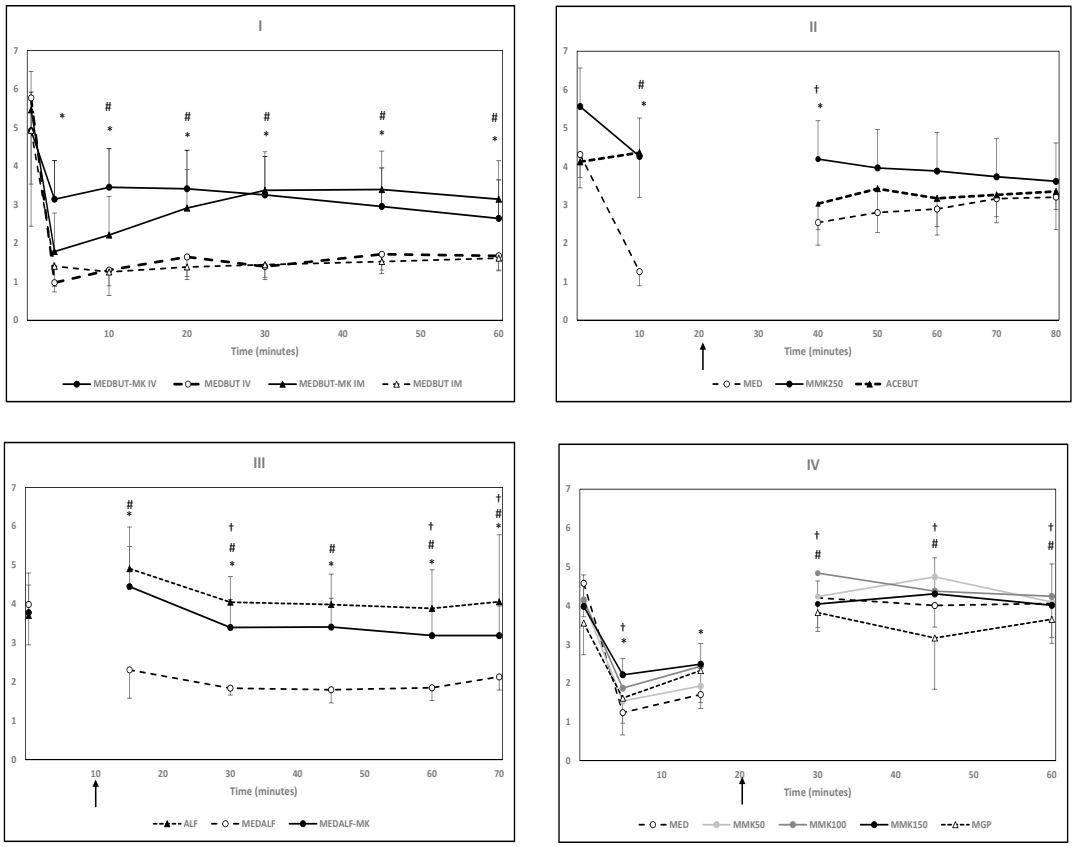

Figure 4. *Mean ± SD cardiac indices (L/min/m²) over time for eight dogs sedated with MEDBUT IV/IM, MEDBUT IV/IM (I); premedicated with MED (II, IV), MMK250 (II), ACEBUT (II), MGP (IV), MMK150 (IV) and MMK50 (IV) prior to isoflurane anaesthesia; or administered a CRI of ALF, MEDALF and MEDALF-MK (See Table 2 for treatment key). The black arrow indicates the time of the induction of anaesthesia with propofol (II), alfaxalone (III) or ketamine and midazolam (IV). * Significant difference between MEDBUT IV and MEDBUT-MK IV (I); MED and MMK250 (II) or MMK150 (IV); or MEDALF and MEDALF-MK (III) ($p < 0.05$). # Significant difference between MEDBUT-IM and MEDBUT-MK IM (I); MED and ACEBUT (II) or MGP (IV); or MEDALF and ALF (III) ($p < 0.05$). † Significant difference between MMK250 and ACEBUT (II); MEDALF-MK and ALF (III); or MMK150 and MGP (IV) ($p < 0.05$). § Significant difference between MMK150 and MMK50 (IV) ($p < 0.05$).*

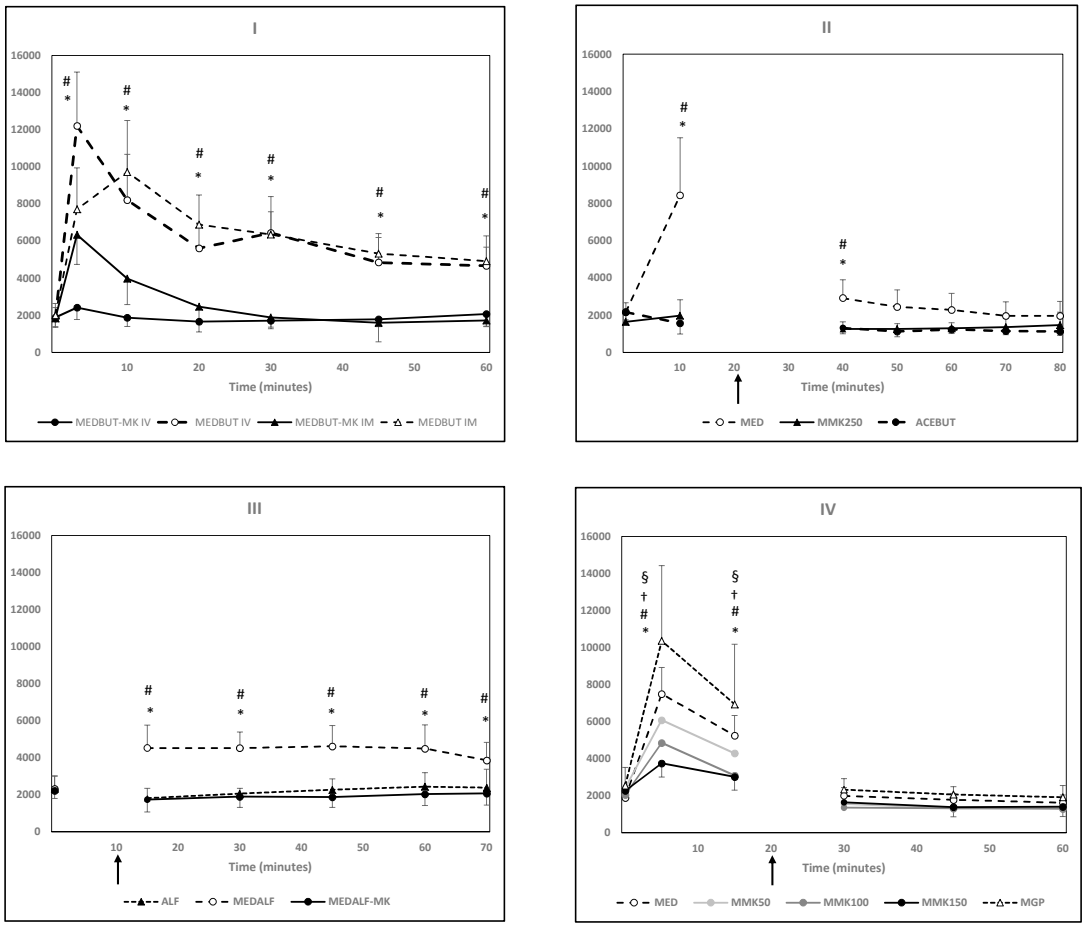

Figure 5. *Mean ± SD systemic vascular resistance indices (dynes*sec*cm^{-5}/m^2) over time for eight dogs sedated with MEDBUT IV/IM, MEDBUT IV/IM (I); premedicated with MED (II, IV), MMK250 (II), ACEBUT (II), MGP (IV), MMK150 (IV) and MMK50 (IV) prior to isoflurane anaesthesia; or administered a CRI of ALF, MEDALF and MEDALF-MK (See Table 2 for treatment key). The black arrow indicates the time of the induction of anaesthesia with propofol (II), alfaxalone (III) or ketamine and midazolam (IV). * Significant difference between MEDBUT IV and MEDBUT-MK IV (I); MED and MMK250 (II) or MMK150 (IV); or MEDALF and MEDALF-MK (III) ($p < 0.05$). # Significant difference between MEDBUT-IM and MEDBUT-MK IM (I); MED and ACEBUT (II) or MGP (IV); or MEDALF and ALF (III) ($p < 0.05$). † Significant difference between MMK250 and ACEBUT (II); MEDALF-MK and ALF (III); or MMK150 and MGP (IV) ($p < 0.05$). § Significant difference between MMK150 and MMK50 (IV) ($p < 0.05$).*

Table 3. *Mean ± SD of SAP, DAP, CVP, DO_2I, PaO_2, $PaCO_2$, $P_{(A-a)}O_2$, RR and rectal temperature in eight dogs sedated with MEDBUT IV/IM and MEDBUT-MK IV/IM (See Table 2 for treatment key).*
** Significant difference between MEDBUT IV and MEDBUT-MK IV ($p<0.05$). # Significant difference between MEDBUT IM and MEDBUT-MK IM ($p<0.05$).*

STUDY I							
Parameter	**Treatment**	**Baseline**	**3**	**10**	**20**	**30**	**60**
SAP (mmHg)	MBMK IV	198 ± 32	211 ± 41 *	150 ± 29 *	130 ± 31 *	124 ± 30 *	126 ± 18 *
	MB IV	191 ± 38	218 ± 16	190 ± 19	173 ± 13	169 ± 17	157 ± 15
	MBMK IM	204 ± 24	221 ± 18	182 ± 20	155 ± 23 #	137 ± 22 #	130 ± 18 #
	MB IM	200 ± 16	209 ± 19	197 ± 9	179 ± 18	173 ± 17	162 ± 22
DAP (mmHg)	MBMK IV	85 ± 12	72 ± 18 *	64 ± 10 *	53 ± 10 *	49 ± 10 *	49 ± 7 *
	MB IV	89 ± 10	127 ± 10	114 ± 6	98 ± 11	94 ± 9	79 ± 13
	MBMK IM	95 ± 14	113 ± 12	87 ± 12 #	68 ± 12 #	64 ± 13 #	51 ± 8 #
	MB IM	88 ± 9	99 ± 16	119 ± 15	102 ± 12	96 ± 13	84 ± 12
CVP (mmHg)	MBMK IV	2 ± 2	6 ± 1	3 ± 1 *	2 ± 1 *	2 ± 2 *	1 ± 2 *
	MB IV	2 ± 3	8 ± 2	11 ± 2	9 ± 2	7 ± 2	6 ± 2
	MBMK IM	2 ± 2	4 ± 3	7 ± 1	4 ± 1 #	3 ± 1 #	2 ± 1 #
	MB IM	2 ± 3	4 ± 2	9 ± 3	9 ± 1	7 ± 1	5 ± 2
DO_2I (mL/min/m^2)	MBMK IV	840 ± 227	395 ± 65 *	500 ± 64 *	495 ± 83 *	471 ± 61 *	382 ± 82
	MB IV	972 ± 519	152 ± 39	253 ± 92	324 ± 121	264 ± 67	307 ± 70
	MBMK IM	920 ± 166	277 ± 57	374 ± 81 #	466 ± 105 #	512 ± 60 #	478 ± 64 #
	MB IM	867 ± 252	238 ± 92	232 ± 113	271 ± 71	282 ± 81	306 ± 68
PaO_2 (mmHg)	MBMK IV	98 ± 4	48 ± 4 *	65 ± 6	73 ± 5	80 ± 5	95 ± 8
	MB IV	98 ± 7	68 ± 5	67 ± 11	77 ± 9	79 ± 9	90 ± 8
	MBMK IM	92 ± 7	70 ± 8 #	73 ± 6	74 ± 6	81 ± 11 #	97 ± 8
	MB IM	97 ± 5	85 ± 5	70 ± 11	71 ± 8	77 ± 9	88 ± 4
$PaCO_2$ (mmHg)	MBMK IV	33 ± 2	40 ± 2 *	43 ± 2 *	44 ± 3 *	44 ± 3 *	42 ± 4 *
	MB IV	34 ± 1	37 ± 2	40 ± 2	38 ± 3	40 ± 3	40 ±3
	MBMK IM	35 ± 2	36 ± 2	38 ± 3	43 ± 2 #	43 ± 3 #	42 ±3 #
	MB IM	34 ± 2	36 ± 2	39 ± 3	40 ± 2	40 ± 3	40 ±2
$P_{(A-a)}O_2$ (mmHg)	MBMK IV	15 ± 4	57 ± 4 *	37 ± 5	28 ± 4	20 ± 4 *	8 ± 5 *
	MB IV	13 ± 7	40 ± 5	38 ± 10	29 ± 8	26 ± 8	15 ± 8
	MBMK IM	19 ± 8	39 ± 8 #	34 ± 5	28 ± 7 #	20 ± 9 #	5 ± 5 #
	MB IM	14 ± 4	24 ± 3	36 ± 8	34 ± 7	28 ± 7	16 ± 4
RR (breaths/min)	MBMK IV	22 ± 7	9 ± 6	7 ± 5	7 ± 4	7 ± 3	8 ± 4
	MB IV	20 ± 3	11 ± 4	8 ± 3	7 ± 2	7 ± 2	9 ± 3
	MBMK IM	23 ± 7	10 ± 4	8 ± 6	6 ± 3	7 ± 3	7 ± 3
	MB IM	28 ± 14	10 ±5	10 ± 5	8 ± 3	8 ± 4	9 ± 5
Temperature (°C)	MBMK IV	38.2 ± 0.5	38.2 ± 0.4	38.0 ± 0.4	37.7 ± 0.5 *	37.5 ± 0.5 *	36.8 ± 0.5 *
	MB IV	38.0 ± 0.3	38.1 ± 0.2	38.0 ± 0.4	38.1 ± 0.3	38.0 ± 0.3	37.5 ± 0.3
	MBMK IM	38.0 ± 0.4	38.1 ± 0.4	38.1 ± 0.3	37.8 ± 0.3 #	37.4 ± 0.5 #	36.9 ± 0.3 #
	MB IM	38.0 ± 0.4	38.2 ± 0.3	38.1 ± 0.3	38.1 ± 0.2	37.9 ± 0.4	37.5 ± 0.4

Table 4. *Mean ± SD of SAP, DAP, CVP, DO_2I, Hb_a, PaO_2, $PaCO_2$, RR, BIS, EMG and rectal temperature in eight dogs premedicated with IV MED, MMK250 or ACEBUT (See Table 2 for treatment key). Anaesthesia was induced 20 minutes later with propofol and maintained with ET_{ISO} 1.2% of isoflurane. * Significant difference between MED and MMK250 (p<0.05). # Significant difference between MED and ACEBUT (p<0.05). † Significant difference between MMK250 and ACEBUT (p<0.05).*

STUDY II						
Parameter	**Treatment**	**Baseline**	**10**	**40**	**60**	**80**
SAP (mmHg)	MED	192 ± 25	184 ± 13	143 ± 13	129 ± 12	122 ± 17
	MMK250	192 ± 17	160 ± 17 *	104 ± 15 *	102 ± 18 *†	100 ± 14 *†
	ACEBUT	194 ± 20	152 ± 29 #	94 ± 26 #	87 ± 15 #	84 ± 13 #
DAP (mmHg)	MED	89 ± 14	114 ± 15	83 ± 15	71 ± 14	63 ± 16
	MMK250	86 ± 14	69 ± 9 *†	58 ± 17 *†	52 ± 14 *†	50 ± 11 *†
	ACEBUT	89 ± 24	56 ± 9 #	41 ± 10 #	39 ± 7 #	39 ± 6 #
CVP (mmHg)	MED	3.5 ± 2.4	10.5 ± 0.9	6.6 ± 1.3	5.1 ± 1.6	4.6 ±1.3
	MMK250	3.6 ± 2.1	4.1 ± 0.6 *†	3 ± 1.1 *	3.1 ± 1.6 *	2.5 ± 1.3 *
	ACEBUT	3.9 ± 1.8	1.9 ± 1.6 #	3.6 ± 1.1 #	3.9 ± 1.4 #	3.4 ± 1.7 #
DO_2I ($mL/min/m^2$)	MED	760 ± 175	256 ± 82	518 ± 137	555 ± 144	592 ± 166
	MMK250	992 ± 359	712 ± 366 *	737 ± 163 *†	676 ± 144	609 ± 129
	ACEBUT	714 ± 66	708 ± 187 #	508 ± 112	512 ± 136	532 ± 91
Hb_a (g/dL)	MED	13.5 ± 0.9	15.9 ± 0.8	13.9 ± 0.7	13.0 ± 0.6	12.6 ± 0.5
	MMK250	13.5 ± 1.2	13.2 ± 0.9 *†	11.9 ± 0.8 *†	11.7 ± 1.0 *†	11.4 ± 0.7 *†
	ACEBUT	13.3 ± 0.7	12.6 ± 0.7 #	11.3 ± 0.3 #	10.8 ± 0.5 #	10.6 ± 0.5 #
PaO_2 (mmHg)	MED	93 ± 9	87 ± 12	555 ± 17	557 ± 23	558 ± 24
	MMK250	94 ± 6	85 ± 7	553 ± 28	568 ± 20	571 ± 20 †
	ACEBUT	95 ± 3	89 ± 5	549 ± 21	556 ± 14	548 ± 15
$PaCO_2$ (mmHg)	MED	35 ± 3	32 ± 4	45 ± 3	44 ± 3	43 ± 4
	MMK250	36 ± 1	39 ± 3 *	44 ± 4 †	43 ± 5 *†	41 ± 5 *†
	ACEBUT	35 ± 2	38 ± 2 #	48 ± 3 #	48 ± 2 #	48 ± 3 #
RR (breaths/min)	MED	18 ± 5	12 ± 5	7 ± 3	9 ± 3	11 ± 3
	MMK250	18 ± 6	10 ± 3	7 ± 3	11 ± 6	13 ± 9 †
	ACEBUT	18 ± 5	12 ± 6	8 ± 5	8 ± 3	9 ± 3
BIS	MED	96.8 ± 2.8	80.1 ± 8.2	57.5 ± 5.1	58.9 ± 3.1	62.6 ± 8.8
	MMK250	97.6 ± 0.5	82.9 ± 4.4	62.1 ± 13.5 †	64.8 ± 15.1	75.8 ± 14.7 *†
	ACEBUT	98 ± 0	87.1 ± 6.2	72.3 ± 15.9 #	69.1 ± 14 #	67.9 ± 14.5
EMG (decibels)	MED	47.1 ± 3.9	37.9 ± 3.4	29.8 ±2.0	30.4 ± 2.5	31.8 ± 2.9
	MMK250	47.1 ± 5.0	38.5 ± 2.2 †	32.8 ± 3.9 †	33.4 ± 4.5	37.6 ± 5.9 *†
	ACEBUT	49.0 ± 4.3	42.5 ± 4.8 #	36.5 ± 4.9 #	34.0 ± 5.2	32.5 ± 4.2
Temperature (°C)	MED	38.0 ± 0.4	38.1 ± 0.4	37.6 ± 0.3	37.4 ± 0.2	37.3 ± 0.3
	MMK250	38.1 ± 0.4	37.8 ± 0.4 *	37.3 ± 0.5 *	37.1 ± 0.5 *	36.9 ± 0.5 *
	ACEBUT	38.0 ± 0.3	37.9 ± 0.4	37.4 ± 0.2	37.1 ± 0.4 #	37.0 ± 0.4 #

Table 5. *Mean ± SD of SAP, DAP, CVP, DO_2I, Hb_a, CaO_2, PaO_2, $PaCO_2$, RR and rectal temperature in eight dogs anaesthetised with either ALF, MEDALF or MEDALF-MK constant rate infusion (See Table 2 for treatment key). * Significant difference between MEDALF and MEDALF-MK ($p < 0.05$). # Significant difference between MEDALF and ALF ($p < 0.05$). † Significant difference between MEDALF-MK and ALF ($p < 0.05$).*

STUDY III							
Parameter	**Treatment**	**Baseline**	**15**	**30**	**45**	**60**	**70**
SAP (mmHg)	ALF	184 ± 23	151 ± 26 #	158 ± 20	165 ± 18	165 ± 18 #	161 ± 19
	MEDALF	190 ± 22	167 ± 13	155 ± 13	155 ± 16	151 ± 13	150 ± 13
	MEDALF-MK	176 ± 20	140 ± 16*	139 ± 15*†	137 ± 16*†	139 ± 14 †	138 ± 15 †
DAP (mmHg)	ALF	81 ± 12	86 ± 14 #	84 ± 13	91 ± 15	90 ± 12	89 ± 11
	MEDALF	82 ± 13	117 ± 11	91 ± 12	88 ± 11	89 ± 11	89 ± 10
	MEDALF-MK	79 ± 11	74 ± 11 *†	61 ± 8 *†	59 ± 7.0 *†	62 ± 8 *†	61 ± 7 *†
CVP (mmHg)	ALF	3.0 ± 2.6	0.9 ± 1.0 #	0.3 ± 1.3 #	0.6 ± 0.9 #	0.3 ± 0.9 #	0.6 ± 1.5 #
	MEDALF	1.9 ± 1.6	7.6 ± 1.1	6.4 ± 2.0	6.4 ± 1.2	6.4 ± 0.7	6.1 ± 0.8
	MEDALF-MK	1.3 ± 1.8	1.4 ± 1.3 *	1.4 ± 0.9 *†	1.5 ± 1.2 *†	1.6 ± 1.2 *†	1.4 ± 1.4 *†
DO_2I (mL/min/m^2)	ALF	642 ± 205	838 ± 191 #	722 ± 122 #	686 ± 127 #	667 ± 183 #	704 ± 313 #
	MEDALF	686 ± 174	452 ± 126	372 ± 40	353 ± 63	357 ± 57	412 ± 69
	MEDALF-MK	654 ± 127	787 ± 193 *	607 ± 139 *†	589 ± 147 *	536 ± 117 *†	537 ± 131 *†
Hb_a (g/dL)	ALF	13.1 ± 0.5	12.4 ± 0.7 #	12.1 ± 0.3 #	11.7 ± 0.4 #	11.6 ± 0.3 #	11.6 ± 0.6 #
	MEDALF	13.1 ± 0.7	14.5 ± 0.8	13.9 ± 0.7	13.5 ± 0.7	13.3 ± 0.6	13.2 ± 0.6
	MEDALF-MK	13.1 ± 0.5	12.4 ± 0.7 *	12.1 ± 0.6 *	12 ± 0.5 *	11.3 ± 0.5 *	11.3 ± 0.4 *
CaO_2 (mL/dL)	ALF	17.2 ± 0.7	18 ± 1.3 #	17.8 ± 0.3 #	17.2 ± 0.5 #	17.1 ± 0.5 #	17.2 ± 0.9 #
	MEDALF	17.3 ± 0.9	19.8 ± 1.3	20.2 ± 1	19.7 ± 1	19.4 ± 0.9	19.3 ± 0.9
	MEDALF-MK	17.3 ± 0.7	17.7 ± 1.1 *	17.8 ± 0.8 *	17.2 ± 0.7 *	16.8 ± 0.6 *	16.8 ± 0.5 *
PaO_2 (mmHg)	ALF	100 ± 5	455 ± 117 #	558 ± 36	542 ± 55	536 ± 76	542 ± 79
	MEDALF	102 ± 3	220 ± 106	537 ± 56	540 ± 54	554 ± 43	545 ± 56
	MEDALF-MK	101 ± 8	362 ± 124 *†	547 ± 24	550 ± 28	537 ± 57	567 ± 23
$PaCO_2$ (mmHg)	ALF	34 ± 2	43 ± 5 #	37 ± 2 #	36 ± 4 #	36 ± 3 #	35 ± 3 #
	MEDALF	34 ± 1	56 ± 5	47 ± 6	46 ± 7	45 ± 3	48 ± 5
	MEDALF-MK	33 ± 3	52 ± 5 †	40 ± 3 *	39 ± 3 *	39 ± 3 *	39 ± 3 *
RR (breaths/min)	ALF	18 ± 6	8 ± 5 #	15 ± 6 #	16 ± 7 #	18 ± 11 #	18 ± 11 #
	MEDALF	19 ± 7	3 ± 3	7 ±2	7 ± 3	7 ± 3	7 ± 4
	MEDALF-MK	18 ± 5	4 ± 2	10 ± 5	10 ± 5 †	11 ± 4 †	11 ± 5 †
Temperature (°C)	ALF	37.9 ± 0.4	37.5 ± 0.3	37.3 ± 0.3	37.1 ± 0.4	37 ± 0.5	36.9 ± 0.5
	MEDALF	37.9 ± 0.7	37.6 ± 0.7	37.4 ± 0.7	37.3 ± 0.6	37.1 ± 0.6	37 ± 0.6
	MEDALF-MK	38 ± 0.4	37.5 ± 0.4	37.2 ± 0.3	37.1 ± 0.2	37 ± 0.3	37 ± 0.2

Table 6. *Mean ± SD of SAP, DAP, CVP, DO_2I, CaO_2, $PaCO_2$ and RR in eight dogs premedicated with MED, MMK50, MMK150 or MGP (See Table 2 for treatment key). Anaesthesia was induced 20 minutes later with ketamine and midazolam and maintained with ET_{iso} 1.2% of isoflurane. *Significant difference between MED and MMK50 or MMK150 ($p < 0.05$). # Significant difference between MED and MGP ($p < 0.05$). † Significant difference between MGP and MMK50 or MMK150 ($p < 0.05$) § Significant difference between MMK50 and MMK150 ($p < 0.05$).*

STUDY IV							
Parameter	**Treatment**	**Baseline**	**5**	**15**	**35**	**45**	**60**
SAP (mmHg)	MED	183 ± 20	203 ± 15	173 ± 11	151 ± 10	138 ± 14	128 ± 13
	MMK50	195 ± 19	194 ± 18†	168 ± 11†	134 ± 15*†	117 ± 18*†	115 ± 17*†
	MMK150	188 ± 10	177 ± 15*†§	157 ± 17†	120 ± 13*†§	108 ± 17*†	104 ± 16*†
	MGP	191 ± 17	262 ± 23 #	241 ± 29#	158 ± 22	135 ± 25	130 ± 26
DAP (mmHg)	MED	87 ± 12	122 ± 8	101 ± 10	94 ± 10	77 ± 12	67 ± 13
	MMK50	91 ± 14	103 ± 9*†	89 ± 11*†	77 ± 18*†	63 ± 17*†	62 ± 15
	MMK150	88 ± 14	87 ± 9*†§	77 ± 9*†	68 ± 11*†	60 ± 13*†	56 ± 12†
	MGP	88 ± 13	170 ± 27#	166 ± 29#	100 ± 16	82 ± 16	74 ± 20
CVP (mmHg)	MED	5.6 ± 2.2	14.8 ± 2.6	12.0 ± 2.5	8.0 ± 1.7	6.3 ± 1.6	5.9 ± 1.6
	MMK50	6.0 ± 1.7	12.6 ± 1.1*†	10.4 ± 1.3*†	6.4 ± 1.4*	5.1 ± 0.8*	4.9 ± 0.6
	MMK150	4.8 ± 2.4	8.6 ± 2.4*†§	7.1 ± 2.6*†§	4.6 ± 1.2*†	4.1 ± 0.6*†	3.9 ± 0.6*
	MGP	6.0 ± 1.5	13.8 ± 1.2	11.4 ± 1.3	7.3 ± 0.7	5.6 ± 0.9	5.3 ± 1.3
DO_2I ($mL/min/m^2$)	MED	780 ± 170	272 ± 61	327 ± 60	865 ± 146	808 ± 97	790 ± 156
	MMK50	723 ± 323	264 ± 77	353 ± 37	838 ± 165	755 ± 91	756 ± 213
	MMK150	666 ± 137	383 ± 71	416 ± 76	730 ± 123	779 ± 158	687 ± 179*
	MGP	600 ± 195	311 ± 137	474 ± 182	791 ± 121#	781 ± 80	731 ± 100#
CaO_2 (mL/dL)	MED	17 ± 1	19 ± 1	19 ± 1	21 ± 1	20 ± 1	20 ± 1
	MMK50	18 ± 1	17 ± 4*†	18 ± 1*†	20 ± 1*†	19 ± 1*†	18 ± 1*†
	MMK150	17 ± 1	17 ± 1*†	17 ± 1*†§	18 ± 1*†§	18 ± 1*†	17 ± 1*†
	MGP	17 ± 1	19 ± 1	20 ± 1 #	21 ± 1	21 ± 1 #	20 ± 1 #
$PaCO_2$ (mmHg)	MED	34 ± 2	34 ± 4	31 ± 3	53 ± 7	46 ± 4	43 ± 4
	MMK50	33 ± 2	33 ± 3	32 ± 3	48 ± 6*	46 ± 5	43 ± 4
	MMK150	33 ± 3	34 ± 4	33 ± 2	44 ± 6*†§	43 ± 5*†	41 ± 3
	MGP	34 ± 2	33 ± 23	32 ± 3	48 ± 8 #	46 ± 4	43 ± 4
RR (breaths/min)	MED	21 ± 5	14 ± 6	10 ± 4	7 ± 2	10 ± 3	10 ± 5
	MMK50	19 ± 4	11 ± 6*	10 ± 6	9 ± 4	9 ± 4	10 ± 4
	MMK150	19 ± 6	12 ± 8	10 ± 6	8 ± 4	8 ± 4	11 ± 5
	MGP	18 ± 3	10 ± 6#	11 ± 7	6 ± 2	8 ± 3	9 ± 3
Temperature (°C)	MED	38.1 ± 0.3	38.1 ± 0.4	38 ± 0.4	37.8 ± 0.4	37.6 ± 0.4	37.5 ± 0.4
	MMK50	38 ± 0.4	38 ± 0.3	37.8 ± 0.3	37.5 ± 0.2	37.3 ± 0.3	37.2 ± 0.3
	MMK150	38 ± 0.4	37.8 ± 0.5	37.7 ± 0.4	37.3 ± 0.3	37.2 ± 0.4	37.2 ± 0.3
	MGP	38 ± 0.2	37.9 ± 0.2	37.7 ± 0.3	37.5 ± 0.2	37.9 ± 0.3	37.3 ± 0.3

6 DISCUSSION

6.1 Haemodynamic effects

The present studies demonstrated the haemodynamic effects of MK-467 in dogs sedated with MED and selected sedative and anaesthetic agents that are commonly used in veterinary practice. Intravenous administration of MK-467 to conscious dogs dose-dependently attenuated or prevented the haemodynamic effects induced by MED when they were administered concomitantly as a bolus for premedication (II, IV). Similar findings have been reported previously in conscious dogs sedated with DMED or MED and various doses of MK-467 (Enouri et al. 2008b; Honkavaara et al. 2008; 2011; Rolfe et al. 2012). The lowest studied dose of MK-467 (50 μg/kg) in relation to the dose of MED (10 μg/kg) (MMK50) resulted in a significantly lower aBP and CVP during both the premedication phase and isoflurane anaesthesia than MED alone was applied, while the SVRI was significantly lower only during the premedication phase (IV). However, MED-induced decreases in HR, CI and DO_2I were prevented only with the highest studied dose of MK-467 (250 μg/kg) in relation to 10 μg/kg of MED (MMK250) (II), while the extent of the reduction in HR and CI was inversely related to the dose of MK-467 with the lower studied doses of MK-467 during the premedication phase (IV). This is in line with other studies which have investigated various dose ratios of MK-467 and DMED or MED administered as an intravenous bolus to the conscious dogs (Honkavaara et al. 2011) or as a CRI during isoflurane anaesthesia (Kaartinen et al. 2014).

The administration of MEDBUT to conscious dogs either intravenously or intramuscularly (I) resulted in a decrease in HR, CI and DO_2I similarly to previous studies (Ko et al. 2000a; Kuo & Keegan 2004). More recently, a nearly 30% decrease in HR was detected after a low dose of MED (1 μg/kg) despite the presence of BUT (0.4 mg/kg) (Girard et al. 2010). The addition of BUT on the sedation regimen with MED and MK-467 resulted in a higher HR, CI and DO_2I and lower aBP, CVP and SVRI than without MK-467 (I), which is in agreement with the previous studies on DMED or MED with MK-467 in conscious dogs (Honkavaara et al. 2011; Rolfe et al. 2012). Although BUT may cause a mild to moderate decrease in HR when administered alone (Sederberg et al. 1981; Trim 1983; Sawyer et al. 1991; Dodam et al. 2004; Ambrisko et al. 2005), it is highly probable that MED was responsible for the initial haemodynamic changes despite the presence of BUT, as also suggested by others (Pypendop et al. 1996).

The haemodynamic changes induced by MED were obtund during isoflurane anaesthesia induced with either propofol (II) or ketamine and midazolam (IV). In more detail, HR, aBPs, SVRI, CI and DO_2I remained at a clinically more acceptable level during isoflurane anaesthesia than during the premedication phase (II, IV). However, a lower HR during isoflurane anaesthesia with MED was accompanied with a tendency towards lower CI and DO_2 than with MMK250 (II). Isoflurane has been demonstrated to cause a dose-dependent decrease in SVR and aBP, which is presumably due to the vasodilatation and myocardial depression (Mutoh et al. 1997). Additionally, several studies describe the influence of halogenated inhalant anaesthetic agents on the haemodynamic function in dogs premedicated with DMED or MED (Kersten et al. 1993; Kuusela et al. 2001b; Gomez-Villamandos et al. 2005; Grasso et al. 2015), and these results are parallel to the findings of studies II and IV. The administration of MK-467 with MED as an IV premedication regimen (II, IV) resulted in lower aBP during isoflurane anaesthesia with each

studied dose of MK-467 than did MED alone (II, IV). The lower studied doses of MK-467 with MED (MMK50-150) (IV) resulted in a clinically more acceptable MAP (approximately 70 mmHg) during isoflurane anaesthesia, whereas hypotension was sometimes observed with the highest studied dose of MK-467 when administered with MED (MMK250) (II). On the other hand, only with the highest studied dose of MK-467 (MMK250), the HR remained higher during isoflurane anaesthesia than with MED (II). Medetomidine is a more selective α_2-adrenoceptor agonist than MK-467, i.e. the α_2: α_1 ratio is 1620:1 and 105:1 for MED (Virtanen et al. 1988) and MK-467 (Clineschmidt et al. 1988), respectively. It is therefore plausible that lower doses of MK-467 were not able to achieve a complete blockade of vascular α_2-adrenoceptors during isoflurane anaesthesia, which was supported by the haemodynamic effects during the premedication phase, as the MED-induced decrease in HR and increase in SVRI were not completely prevented with these lower doses of MK-467 (IV). In addition to the blockade of peripheral α_2-adrenoceptors, the highest dose of MK-467 (MMK250) may also have induced at least a minor α_1-adrenoceptor blockade as it is more α_1-selective than MED. Based on *in vitro* studies of isolated canine large arteries, such as the aorta and femoral artery, α_1-adrenergic receptors mediate contractile responses exclusively (Kwan 1999; Guimaraes & Moura 2001). Therefore, with the presence of isoflurane, a potent vasodilator, the higher HR with MMK250 than with MED could have been a consequence of lower aBP during isoflurane anaesthesia, leading to the baroreceptor-mediated response due to the relative hypovolemia. On the other hand, the induction agents may also have altered the haemodynamic effects of MK-467 during isoflurane anaesthesia (II, IV). Significant differences in HR were not detected between the lower studied doses of MK-467 (MMK50-150) and MED during isoflurane anaesthesia, but HR was actually slightly higher after the induction with ketamine-midazolam (IV) than after propofol induction (II). Therefore, a slightly higher HR in study IV could also have facilitated the maintenance of the clinically more acceptable MAP. Higher HR, CI and MAP after the induction of anaesthesia with ketamine and midazolam in comparison to propofol have been reported in dogs premedicated with MED (Hellebrekers & Sap 1997; Hellebrekers et al. 1998; Enouri et al. 2008a).

Alfaxalone CRI was accompanied with an increase in HR and the CI and, subsequently, in the DO_2I (III), as also reported in previous studies (Muir et al. 2008; Rodrigues et al. 2012; Amengual et al. 2013; Okushima et al. 2015). As expected, MEDALF induced an increase in the SVRI and MAP, followed by a decrease in HR, CI and DO_2I (III). Similar results were reported during DMED and ALF TIVA in dogs (Quiros Carmona et al. 2014). Decreasing the dose of DMED improved the HR and CI during DEMD-ALF TIVA (Quiros Carmona et al. 2014). The addition of a MK-467 CRI counteracted these changes induced by MEDALF CRI, resulting in stable haemodynamic function (III). In a clinical trial comparing ALF TIVA and isoflurane anaesthesia for Caesarean section in bitches, MAP was significantly lower during isoflurane anaesthesia than during ALF TIVA (Conde Ruiz et al. 2016). Similarly, with these studied doses of anaesthetic drugs, CRI with MEDALF (III) resulted in a higher SVRI and lower HR and CI compared to MED (II, IV) during isoflurane anaesthesia induced with either propofol (II) or ketamine midazolam (IV). This could be due to different actions on vasomotor tone by the used anaesthetic agents *per se*, or to higher actual plasma DMED concentrations achieved with MEDALF (III) than with MED during isoflurane anaesthesia (IV).

The overall haemodynamic outcome was fairly similar between the premedications with MMK and ACEBUT prior to and during isoflurane anaesthesia induced with propofol (II).

However, ACEBUT resulted in consistently lower MAP than MMK250. Additionally, the HR was significantly higher with MMK250, and both CI and DO_2I tended to be higher than with ACEBUT during isoflurane anaesthesia, but the SVRI did not differ between these two premedication regimens (II). The tendency towards a higher CI during isoflurane anaesthesia with MMK250, was the most probably related to the significantly higher HR with MMK250 than with ACEBUT (II). Heart rate with ACEBUT did not differ from MED during isoflurane anaesthesia (II). This is in contrast to the findings reported by Grasso et al. (2015), where ACP alone resulted in a higher HR than DMED during isoflurane anaesthesia induced with propofol. As mentioned earlier, BUT may induce a mild decrease in HR in dogs (Sederberg et al. 1981; Trim 1983; Sawyer et al. 1991; Dodam et al. 2004; Ambrisko et al. 2005). Therefore, the BUT component of ACEBUT could have contributed to the lower HR and, subsequently, have an impact on the lower MAP recorded during isoflurane anaesthesia with ACEBUT premedication (II).

Anticholinergics, e.g., glycopyrrolate or atropine, are effective in preventing bradycardia induced by an α_2-receptor agonist (Vainio & Palmu 1989; Short 1991; Alibhai et al. 1996; Ko et al. 2001b; Congdon et al. 2011). However, a subsequent dramatic increases in blood pressure occurs, which, in combination with normo- or tachycardia, will lead to significantly increased rate pressure product (Congdon et al. 2011), which in turn represents an estimation of the oxygen consumption of the myocardium (Katz & Feinberg 1958). Comparable findings were recorded after pre-treatment with subcutaneous glycopyrrolate and MED prior to isoflurane anaesthesia (IV). Moreover, similarly to the results of Congdon et al. (2011), the CI was not improved in relation to the increased HR with MGP (IV). Additionally, during the isoflurane anaesthesia induced with ketamine and midazolam, the CI was significantly lower with MGP than MED (IV). This is in contrast with previous studies on romifidine-sedated (Sinclair et al. 2002) or isoflurane-anaesthetised dogs (Bloor et al. 1992), which could be due to a different study design, i.e. the dogs were anaesthetised with isoflurane during the entire observation period (Bloor et al. 1992), or due to the use of a less-selective α_2-adrenoceptor agonist, romifidine (Sinclair et al. 2002).

In the present studies, cardiac output was measured by the lithium dilution method (LiDCO) that has been reported to correlate well with the thermodilution method (Mason et al. 2001). A standard dose of lithium chloride was injected prior to each measurement, and no more than seven lithium chloride injections were given during a trial. Based on the lithium pharmacokinetics in beagle dogs (Rosenthal & Koritz 1989), it could be suggested that the background lithium concentration did not probably cause any bias in our studies, as it has been proposed that 34 injections during 3–7 hours are needed to reach a plasma lithium concentration of 0.2 mmol/L (Mason et al. 2002). This plasma lithium concentration is considered by the manufacturer to be the limit for possible overestimation of cardiac output by using the LiDCO method. Additionally, the estimation of cardiac output by means of pulse power analysis for CO (PulseCO) was not used in these studies, as it has been demonstrated that large changes in systemic vascular resistance may have an impact on the accuracy of the results of pulse power analysis and recalibration with lithium chloride dilution is often needed (Pittman et al. 2005; Bein et al. 2007; Duffy et al. 2009; Morgaz et al. 2014). As the control treatment in all studies was MED alone (II, III, IV) or combined with BUT (I), significant changes in SVRIs were expected. Certain sedative and anaesthetic agents, such as xylazine and ketamine, have been demonstrated

to interact with the lithium sensor, causing bias in LiDCO measurements (Ambrisko et al. 2013). Dexmedetomidine interact with the sensor *in vitro*, but the concentrations of dexmedetomidine causing relevant bias (Ambrisko et al.2013) were well above the plasma concentrations of DMED detected in the studies III and IV, as well as in other studies where clinically relevant doses of MED were administered in dogs (Kuusela et al. 2000). Interaction with the LiDCO sensor has not been detected or it is minor with atipametsole, acepromazine, butorfanol or midazolam *in vitro* (Ambrisko et al. 2013). However, the interaction of propofol, alfaxalone, isoflurane, glycopyrrolate or MK-467 with the LiDCO sensor is not known.

In general, DO_2I followed the trends of CI in all studies (I–IV). However, as DO_2I is dependent on both CI and CaO_2, changes in CaO_2 may also have had an impact on DO_2I. In studies II–IV, MK-467 alleviated medetomidine-induced increases in Hb_a and CaO_2. Similar findings have also been reported previously after the administration of an α_2-adrenoceptor agonist (Sinclair et al. 2002; Lin et al. 2008; Pascoe 2015) and when it is combined with MK-467 (Kaartinen et al. 2014). However, it is possible that repeated blood sampling during the trials, i.e. LiDCO measurements and blood sampling for the analyses of blood gases as well as plasma drug concentrations, might have had an influence on the haemoglobin concentration and thus also on other cardiopulmonary parameters (CaO_2, DO_2I). The approximated blood loss during each trial was calculated beforehand, so that the maximum blood sampling would be less than 10% of the blood volume, with 14 days between trials (Diehl et al. 2001). We also aimed to replace the blood loss during the trial to prevent hypovolemia. Therefore, fluid therapy might also enhance haemodilution − e.g., a decrease in haemoglobin − and it might thus have had an effect on the results, similarly to the blood loss during the studies. However, the differences detected between the various treatments could be considered to be reliable, as the blood loss and fluid administration were similar between the treatments.

6.2 Respiratory effects and blood gases

As expected, the RR decreased after all treatments and was accompanied with a moderate decrease in PaO_2 and an increase in $PaCO_2$, although no severe acidosis (pH <7.2) was detected.

Whilst the haemodynamic outcome after the sedation with MEDBUT-MK administered via the IV or IM route was comparable to those studies conducted without an opioid (Honkavaara et al. 2011; Rolfe et al. 2012; Restitutti et al. 2017), the presence of BUT with MED and MK-467 probably affected on the arterial oxygenation in dogs breathing room air (I). Combining MK-467 with MEDBUT did not affect RR, but shortly after the administration of MEDBUT-MK IV or IM, a transient but clinically relevant and statistically significant decrease in PaO_2 was detected (I). The decrease in PaO_2 was more pronounced after IV than IM administration (I). This is in contrast to studies where dogs were sedated with DMED and MK-467 without an opioid component (Enouri et al. 2008b; Honkavaara et al. 2011; Rolfe et al. 2012; Restitutti et al. 2017). The decrease in PaO_2 was accompanied with a significant increase in $P_{(A-a)}O_2$ (I). The less-intense changes in $PaCO_2$ in relation to PaO_2 would suggest that the reason for the reduction of PaO_2 was related to a ventilation-perfusion mismatch rather than hypoventilation *per se*. Nevertheless, the better-maintained CI with MEDBUT-MK seemed to compensate for the transient decrease in PaO_2 and subsequent decrease in CaO_2, ensuring a better DO_2I than with MEDBUT (I).

As expected, the hypoventilation was the most evident after the induction of anaesthesia with propofol (II) or ketamine-midazolam (IV), which is comparable to several studies where dogs were premedicated with MED or ACP and induced with propofol or ketamine (Hellebrekers & Sap 1997; Hellebrekers et al. 1998; Kuusela et al. 2001b; Enouri et al. 2008a; Grasso et al. 2015). During the anaesthetic phases (II, III, IV) the dogs were breathing nearly 100% of oxygen, resulting in a high PaO_2. MED has been demonstrated to decrease the sensitivity of the respiratory centre and the central ventilatory drive in response to an increase in $PaCO_2$ in conscious dogs (Lerche & Muir 2004). MED may also augment the respiratory depression induced by other agents, such as isoflurane (Lerche & Muir 2006). Likewise, in the present study (III) hypoventilation was the most evident in a MEDALF CRI, but the addition of MK-467 attenuated these changes. Similar trends in $PaCO_2$ were noticed during isoflurane anaesthesia with increasing doses of MK-467 (II, IV), although a statistically significant difference during isoflurane anaesthesia was reached only with the highest studied dose of MK-467 (MMK250) (II). However, the addition of MK-467 did not influence the changes in RR during isoflurane anaesthesia (II, IV). The differences in the depth of anaesthesia (II, III) or in plasma DMED and ALF concentrations (III) between treatments with and without MK-467 may have contributed to the extent of hypoventilation. Nevertheless, during isoflurane anaesthesia, $PaCO_2$ was the highest when ACEBUT had been used for premedication (II). Grasso et al. (2015) demonstrated that premedication with ACP induced less hypoventilation than premedication with MED during isoflurane anaesthesia induced with propofol. Additionally, BUT and ACP had a higher incidence of apnoea after propofol induction than ACP alone (Bufalari et al. 1997). On the other hand, the dose of propofol needed for induction after ACEBUT (II) might also have had an influence on the magnitude of hypoventilation, as propofol has been demonstrated to have dose-dependent ventilatory depressant effects in dogs (Keates & Whittem 2012). Thus, the hypoventilation related to ACEBUT during isoflurane anaesthesia may have been due to the BUT, the induction dose of propofol or both.

During the present studies, the dogs were breathing spontaneously for the evaluation of the effects of MK-467 on ventilatory function under the influence of other sedative and anaesthetic agents. Therefore, the detected differences in $PaCO_2$ between the various treatments may have had a subtle influence on haemodynamic function. Hypercapnia has direct negative effects on the myocardium, while CO_2 indirectly affects on the cardiovascular system by stimulating the sympathetic nervous system (Feihl & Perret 1994). The stimulation of the sympathetic nervous system usually overrides the direct effect of CO_2 on the myocardium, resulting in an increase in HR and CI, and reduction in a SVR, without influencing MAP (Arantxa et al. 2000). Nevertheless, the hypoventilation during the studies were in the limits of permissive hypercapnia i.e. less than 60 mmHg, and variation between treatments in $PaCO_2$ were less than 10 mmHg. To keep $PaCO_2$ similar between treatments would have needed to induce intermittent positive pressure ventilation after the induction of anaesthesia, which in turn, could also have affected haemodynamic function by decreasing venous return and subsequently cardiac output (Kyhl et al. 2013). Furthermore, the lateral recumbency of the dogs during the studies may also have affected the PaO_2 and $PaCO_2$ when the dogs were breathing room air. The PaO_2 in conscious dogs has been demonstrated to be significantly higher in sternal than in lateral recumbency (McMillan et al. 2009). Additionally, atelectasis has been detected in sedated dogs after 15 minutes in lateral recumbency (Barletta et al. 2014).

6.3 Plasma concentrations of drugs (III, IV)

The plasma DMED concentrations were significantly lower when MK-467 was co-administered with MEDALF as a CRI (III) or as an IV bolus with the dose of 150 µg/kg with MED (MMK150) prior to isoflurane anaesthesia (IV). This agrees with other studies on conscious dogs (Honkavaara et al. 2012; Bennett et al. 2016). In addition to its effects on plasma DMED concentration, MK-467 also reduced ALF plasma concentrations during MEDALF-MK CRI (III). Currently, it is not known whether MK-467 influences the pharmacokinetics of other sedative or anaesthetic agents, such as butorphanol, in dogs when they are co-administered intravenously with MED. In horses, concurrent IV administration of MK-467 reduced plasma concentrations of both detomidine and butorphanol and resulted a smaller AUC_{last} (Pakkanen et al. 2015).

The lower plasma DMED concentration after the co-administration of DMED and MK-467 has been suggested to be related to the increase in the early disposition of DMED due to the better-maintained CI and tissue perfusion with this treatment than with DMED alone (Honkavaara et al. 2012). In addition, atipamezole has been reported to increase the clearance of MED, resulting in a lower AUC of MED in comparison to MED alone (Salonen et al. 1995). Similarly, an MK-467 CRI had a significant effect on the systemic clearance of DMED in dogs anaesthetised with a MED CRI and isoflurane (Kaartinen et al. 2014). The attenuation of α_2-agonist-induced haemodynamic effects with an α_2-adrenoceptor antagonist most likely ensured the restoration of hepatic perfusion in these studies. Moreover, the ALF plasma concentration during the MEDALF CRI (III) was almost twice as high as with an ALF CRI alone (III). DMED has been demonstrated to reduce its own clearance by decreasing cardiac output (Dutta et al. 2000). Therefore, it could also be suggested that an increase in the ALF plasma concentration with MEDALF was also a consequence of the reduction in CI and tissue perfusion with this treatment. On the other hand, the impact of DMED on liver blood flow through the hepatic artery as measured with the radioactive microsphere method was unaffected in dogs anaesthetised with chloralose-urethane or fentanyl-halothane (Lawrence et al. 1996). However, to the author's knowledge, it is not currently known whether hepatic perfusion would be similarly unaffected after the administration of DMED or MED in conscious dogs or in the presence of other anaesthetic agents. Nevertheless, the prevention or attenuation of the MED-induced decrease in cardiac output with MK-467 probably altered the early disposition of DMED and ALF and maintained liver perfusion, leading to lower DMED and ALF plasma concentrations (III, IV).

In contrast to IV administration, IM administration of MED and MK-467 has been demonstrated to lead to higher early-stage plasma DMED concentrations than MED alone (Restitutti et al. 2017). This was suggested to be due to the enhanced absorption of MED when MK-467 is administered concomitantly (Restitutti et al. 2017). MK-467 most likely prevents MED-induced vasoconstriction in the muscle and thus hasten medetomidine absorption from the injection site. Preliminary results also suggest that MK-467 may enhance the absorption of BUT when administered concomitantly IM with MED and MK-467 (Kallio-Kujala et al. 2017). It could therefore be suggested that, also in the present study, the IM administration of MEDBUT-MK (I) lead to high initial plasma concentrations of DMED and BUT, which was seen as a significant but transient decrease in HR and CI after this treatment. In addition, the time for peak plasma concentration has been detected to be significantly later for MK-467 than DMED (Restitutti et al. 2017).

The blood samples for the analysis of plasma drug concentrations were taken from a central venous catheter. It should also be kept in mind that measured plasma drug concentrations might differ depending on the sampling site, i.e. between venous or arterial samples (Hedges et al. 2013). In studies III and IV, the first plasma sampling was obtained at 5 (IV) and 15 (III) minutes after the IV administration of the drugs. At these time points, there were more deviations in the plasma drug concentrations with MED (IV) or MEDALF (III) treatments in comparison to treatments including MK-467. This might be the result of an unequal dilution of the drugs in the central compartment with MED and MEDALF treatments. Arterial blood sampling might therefore have resulted in more accurate plasma concentrations.

6.4 The level of sedation, BIS and doses of induction agents

The level of sedation was assessed using a subjective CSS by a blinded observer, and BIS recordings (II). At the time of induction, no difference in CSS was detected between MED and MMK250 or ACEBUT (II). This is in concert with previous studies where MK-467 did not influence the sedation score in dogs sedated with IV DMED or MED (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012; Bennett et al. 2016). Although BIS was lower with MED than with MMK250 or ACEBUT (II), no differences were detected before the induction in BIS or in EMG recordings between MED and MMK250 (II). Therefore, it could be suggested that the level of sedation at the time of the induction was rather similar between MED and MMK250 (II). In an earlier study with sedated dogs, BIS was similarly lower with DMED alone than with MK-467 (Restitutti et al. 2011). The lower BIS with DMED or MED alone in comparison to the presence of MK-467 was most likely influenced by differences in plasma DMED concentrations between the treatments (IV; Honkavaara et al. 2012). However, plasma drug concentrations were not measured in study II, and hence this assumption cannot be confirmed. During the constant isoflurane anaesthesia, ET_{ISO} 1.2% (II, IV), all dogs seemed to be in the surgical plane of anaesthesia based on a subjective clinical evaluation. Arguably, the ET_{ISO} could have been lower, as a DMED CRI has been demonstrated to cause a significant decrease in the MAC of both isoflurane (Pascoe et al. 2006; Ebner et al. 2013; Pascoe 2015) and sevoflurane (Moran-Munoz et al. 2014; Hector et al. 2017) in dogs. However, the knowledge of the influence of MK-467 on the MAC of inhalant agents in dogs was not available at the time when the studies were conducted. Recently, MK-467 has been demonstrated to increase sevoflurane MAC in dogs (Hector et al 2017). It is highly probable that this is also true in the case of isoflurane. Therefore, the depth of anaesthesia might have been deeper with MED than with the treatments including MK-467 (II, IV), which was supported by the results from the BIS recordings (II). Previously, it has been demonstrated that BIS correlates well with the MAC multiples of sevoflurane in dogs (Greene et al. 2002). In addition, in surgical canine patients, BIS was able to differentiate a light level of anaesthesia from a surgical level (Bleijenberg et al. 2011). On the other hand, muscular activity may interfere with BIS recordings (Bruhn et al. 2000), which might also have an impact on the differences detected between the treatments (II). Nevertheless, differences in the anaesthetic depths between treatments might also have affected haemodynamic data obtained from studies II and IV.

Premedication with MED resulted in lower induction doses of propofol than with MMK250 or ACEBUT (II). The quality of induction was smooth, and the extent of hypoventilation

did not differ between MED and MMK250, despite the lower propofol dose with MED (II). Medetomidine has been demonstrated to decrease the volume of distribution of propofol in dogs (Hall et al. 1994). In addition, concomitant IV administration of DMED or MED and MK-467 resulted in lower plasma DMED concentrations, a higher volume of distribution and faster clearance of DMED than the corresponding dose of DMED or MED alone (Honkavaara et al. 2012; Bennett et al. 2016). Therefore, it is possible that the plasma propofol concentration was similarly influenced by MK-467, resulting in higher propofol dosing (II). As mentioned earlier, plasma drug concentrations were not measured in this study (II), and hence this assumption cannot be confirmed.

On the contrary, the addition of MK-467 to MED did not influence the induction doses of ketamine and midazolam (IV). Similarly, in horses premedicated with detomidine and BUT with and without MK-467, the anaesthesia was induced with a standard dose of ketamine and midazolam with no differences in plasma ketamine or midazolam concentrations between the treatments (Pakkanen et al. 2015). Plasma ketamine and midazolam concentrations were not measured in the present study (IV), so we cannot confirm that this is also true in dogs. Nevertheless, the horses were induced faster with than without MK-467 (Pakkanen et al. 2015), which was presumably due to the better cardiac output with MK-467, resulting in faster drug delivery to the CNS than without MK-467. We did not detect any difference in the speed of induction regardless of the premedication regimen (IV), most probably due to the different dosing of induction agents (ketamine and midazolam) in our study (IV) than the study in horses (Pakkanen et al. 2015). We administered ketamine and midazolam with various increments over time (IV), while the horses where induced with fast boluses of induction agents (Pakkanen et al. 2015).

Dogs that were sedated with MEDBUT with or without MK-467 (I), received atipamezole 50 μg/kg IM at end of the study. Although recovery quality or time was not assessed by using a blinded observer, all dogs recovered smoothly with no signs of excitement or other clinically detectable side effects. This is a comparable finding to other studies where atipamezole has been administered to reverse sedation induced by MED and MK-467 in dogs (Honkavaara et al. 2008; Turunen et al. 2015).

6.5 Rectal temperature

Impaired thermoregulation is a common side effect in sedated or anaesthetised animals, and additional efforts are needed, which is why safe heat supplementation is mandatory in clinical practice to prevent hypothermia. During studies II–IV, dogs were kept on an isolating mattress and an electrical heating pad, and covered with blankets, aiming to maintain normothermia. During study I, dogs were kept on an isolating mattress, but no further efforts were made, as data were collected simultaneously for a thermographic imaging study (Vainionpää et al. 2013a).

Anaesthetic and sedative drugs interfere with thermoregulation via several mechanisms, such as impairing hypothalamic thermoregulation responses, vasodilatation and decrease in sympathetic outflow (Diaz & Becker 2010). The present studies demonstrated, that by modifying haemodynamic function by blocking peripheral α_2-adrenergic receptors with MK-467, heat loss may also be altered during sedation (I) and general anaesthesia (II).

In dogs sedated with MEDBUT, the superficial temperature measured by thermography declined, while the addition of MK-467 resulted in an increase in superficial temperature (Vainionpää et al. 2013a). The α_2-adrenoceptors are distributed distally in the peripheral arteries of the extremities (Flavahan et al. 1987), and the activation of α_2-adrenergic receptors has been demonstrated to result in a decrease in skin blood flow in mice (Honda et al. 2007). Additionally, in canine cutaneous resistance arteries, α_{1A}- receptors are also involved in vasoconstriction (Argyle & McGrath 2000). It could therefore be suggested that a blockade of α-adrenoceptors with MK-467 prevented vasoconstriction induced by MED, and augmented the superficial blood flow followed by heat loss via an increased convection from core to surface (I, II).

Severe hypothermia (29°C) decreases tissue metabolic activity and oxygen consumption followed by reducing HR and CI (Morray & Pavlin 1990), while oxygen consumption is increased during rewarming and shivering (Ralley et al. 1988; Morray &Pavlin 1990). Moderate hypothermia (approximately 32°C) has been induced in pigs with DMED and isoflurane anaesthesia when supplemental heating was not provided (Vainio & Bloor 1994). However, aBP or HR were not affected (Vainio & Bloor 1994). Thus, the decline in rectal temperature leading to mild hypothermia in the present studies could be considered to cause minor effects on haemodynamic function.

6.6 Practical relevance and future prospects

The present studies were aimed to evaluate the haemodynamic effects of MK-467 in combination with MED and selected sedative and anaesthetic agents in dogs. The adjunctive drugs used for sedation or to induce and maintain anaesthesia were chosen based on their common use in clinical veterinary practice. Additionally, the studies were designed so that the order of drug administration would mimic clinical settings and thus would facilitate the transfer of MK-467 to clinical practice. Based on these studies, MK-467 would extend the clinical safety margin of MED as a part of the sedative or premedication regimen in healthy dogs. However, as the current studies were experimental in nature and no surgical stimulus was applied, a prospective, randomized clinical trials with client-owned healthy dogs are warranted. In addition, as maintaining anaesthesia with isoflurane in these studies (II and IV) predisposed to rather low arterial blood pressures in dogs premedicated with MK-467 and MED, further studies are needed to evaluate the influence of MK-467 on the efficacy of certain sympathomimetic drugs, such as dopamine or dobutamine.

The present studies demonstrated that the concomitant administration of MK-467 with MED influences the plasma concentration of DMED (III, IV) and ALF (III), most probably by affecting the early disposition of DMED (Honkavaara et al. 2012; Bennet et al. 2016). In the present studies (III, IV), plasma DMED concentrations in the presence of MK-467 during the anaesthetic phases were less than 2 ng/mL, which has been reported as a minimum plasma concentration of DMED associated with antinociception in dogs (Van Oostrom et al. 2011). Therefore, this decrease in the plasma concentration of DMED or other agents might have an impact on the quality of analgesia or sedation or on the maintenance of general anaesthesia in clinical situations where the patient may experience preoperative pain or undergo a surgical procedure. Therefore, further evaluation of the influence of MK-467 on the analgesic and anaesthetic sparing effects of MED are needed in experimental or clinical settings where constant

plasma DMED concentrations are applied. Later, after the clinical studies in healthy dogs, an assessment of the suitability of the combination of MK-467 and MED in variously compromised patients could be taken into a consideration, as these patients may also benefit from the desired effects of MED.

7 CONCLUSIONS

1. MK-467 attenuated the haemodynamic effects in dogs sedated with medetomidine and butorphanol. The beneficial effects of MK-467 appeared more slowly after intramuscular than after intravenous administration.

2. When administered as an intravenous bolus, MK-467 dose-dependently attenuated the haemodynamic effects of medetomidine during the preanaesthetic phase, but not during inhalation anaesthesia with isoflurane induced with either propofol or ketamine-midazolam.

3. Premedication with MK-467 and medetomidine mimics the overall haemodynamic effects of premedication with acepromazine/butorphanol prior to and during isoflurane anaesthesia. Medetomidine-induced peripheral systemic changes were attenuated by MK-467 and augmented by glycopyrrolate. Pre-treatment with subcutaneous glycopyrrolate was not able to improve the medetomidine-induced reduction in cardiac output by the means of increasing the heart rate prior to or during isoflurane anaesthesia induced with ketamine and midazolam.

4. A concomitant constant rate infusion of MK-467, medetomidine and alfaxalone produced a stable haemodynamic outcome. Medetomidine-induced haemodynamic changes were restored with MK-467 during alfaxalone total intravenous anaesthesia.

5. Intravenously administered MK-467 resulted in reductions in plasma dexmedetomidine and alfaxalone concentrations, presumably by altering the haemodynamic function.

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