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Dexmedetomidine and MK-467, a peripherally acting α_2-adrenoceptor antagonist, in dogs

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ACADEMIC DISSERTATION

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’ To my parents ’

Abstract

The effects of MK-467, a peripherally acting α_2-adrenoceptor antagonist, on the cardiopulmonary changes induced by dexmedetomidine, a specific and selective α_2-adrenoceptor agonist, were investigated in dogs. Plasma concentrations of both drugs were also quantified, along with influence of MK-467 on the quality of clinical sedation achieved with dexmedetomidine.

The main focus of this study was on preventing or attenuating the cardiovascular effects of dexmedetomidine. The effects of three different doses of MK-467, administered simultaneously with the agonist, on the main hemodynamic parameters were evaluated and compared with a single dose of both dexmedetomidine and MK-467 administered alone. Respiratory effects were evaluated with arterial blood gases, and indices such as oxygen delivery were calculated. The pharmacokinetic interaction between the two drugs was evaluated *in vivo* and general information on the disposition of intravenously administered MK-467 in dogs was produced. The degree of clinical sedation was subjectively assessed during the studies. The effect of MK-467 on the quality of reversal of dexmedetomidine-induced sedation with atipamezole was also determined.

MK-467 dose-dependently reduced or prevented all relevant cardiovascular changes induced by dexmedetomidine. The heart rate, arterial and central venous blood pressure, cardiac index, systemic vascular resistance and oxygen delivery remained within acceptable physiological limits throughout the observational period with all doses of MK-467 administered with the agonist. Moderate hypotension was seen with the highest dose of MK-467. No significant differences in respiratory function were observed between treatments. MK-467, when administered alone, induced sinus tachycardia along with increases in the cardiac index and reductions in systemic vascular resistance. Arterial blood pressures remained unchanged.

The degree of clinical sedation was reduced by MK-467, most likely by increasing the disposition of dexmedetomidine, which led to lower plasma concentrations of the agonist. However, the differences in clinical sedation were minor. MK-467 did not interfere with the ability of atipamezole to reverse dexmedetomidine-induced sedation.

List of original publications

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

I

Honkavaara JM, Raekallio MR, Kuusela EK, Hyvärinen EA, Vainio OM. The effects of L-659,066, a peripheral a2-adrenoceptor antagonist, on dexmedetomidine-induced sedation and bradycardia in dogs. *Veterinary Anaesthesia and Analgesia*, 2008, 35, pp 409-413.

II

Honkavaara JM, Restitutti F, Raekallio MR, Kuusela EK, Vainio OM. The effects of increasing doses of MK-467, a peripheral alpha2-adrenergic receptor antagonist, on the cardiopulmonary effects of intravenous dexmedetomidine in conscious dogs. *Journal of Veterinary Pharmacology and Therapeutics*, 2010, 34, pp 332-337.

III

Restitutti F, Honkavaara JM, Raekallio MR, Kuusela EK, Vainio OM. Effects of different doses of L-659'066 on the bispectral index and clinical sedation in dogs treated with dexmedetomidine. *Veterinary Anaesthesia and Analgesia*, 2011, 38, pp 415-422.

IV

Honkavaara J, Restitutti F, Raekallio M, Salla K, Kuusela E, Ranta-Panula V, Rinne V, Vainio O, Scheinin M. Influence of MK-467, a peripherally acting α2-adrenoceptor antagonist on the disposition of intravenous dexmedetomidine in dogs. *Drug Metabolism and Disposition*, 2012, 40, pp 445-449.

Abbreviations

ADH	antidiuretic hormone
ATP	adenosine triphosphate
AUC	area under curve
BBB	blood–brain barrier
cGMP	cyclic guanosine 3', 5' monophosphate
CI	cardiac index
CI95	95% confidence interval
CNS	central nervous system
CO	cardiac output
CSS	composite sedation score
CVP	central venous pressure
DAP	diastolic arterial pressure
DO_2	oxygen delivery
ECG	electrocardiography
GABA	gamma-amino butyric acid
HR	heart rate
IM	intramuscular
IUPAC	international union of pure and applied chemistry
IV	intravenous
MAC	minimal alveolar concentration
MAP	mean arterial pressure
m/z	mass-to-charge ratio
NLC	nucleus locus coeruleus
N_2O	nitrous oxide
$P(A\text{-}a)O_2$	alveolar-to-arterial oxygen partial pressure difference
$PaCO_2$	arterial partial pressure of carbon dioxide
PaO_2	arterial partial pressure of oxygen
SaO_2	arterial hemoglobin saturation percent
SAP	systolic arterial pressure
SD	standard deviation
SVR	systemic vascular resistance
Vd	volume of distribution

1. Introduction

Dexmedetomidine is a potent and specific alpha$_2$-adrenoceptor agonist that is widely used in small animal practice as a sedative and/or pre-anesthetic drug. Its use is currently limited in compromised animals due to characteristic effects on the cardiovascular system, including hypertension, bradycardia, and consequent decreases in the cardiac index and tissue oxygen delivery. When necessary, both the central and peripheral outcomes can be reversed with atipamezole, a specific alpha$_2$-adrenoceptor antagonist.

MK-467 (also known as L-659,066) is an alpha$_2$-adrenoceptor antagonist that is thought not to cross the mammalian blood–brain barrier, limiting its pharmacodynamics to peripheral organ systems. MK-467 has gained increasing interest due to its potential for preventing or attenuating the peripheral hemodynamic effects of both medetomidine and dexmedetomidine, while allowing for the central properties of the agonist drugs.

Thus far, the agonist–antagonist combination has been preliminarily studied in dogs and sheep, without reported adverse effects and showing promising stability of cardiovascular function.

2. Review of the literature

2.1 Adrenergic receptors

Adrenergic receptors are an essential part of the autonomic nervous system, mediating their main physiological effects via adrenaline and noradrenaline. Adrenoceptors are classified into types, α and β, depending on their specific location and action (Ahlquist, 1948). The α-adrenoceptors are then further divided into types α_1 and α_2 according to their specific physiological properties along with varying affinities to both endo- and exogenous ligands. Type α_1-adrenoceptors mediating the effects of catecholamines are mainly located on the post-synaptic membrane whereas the role of the mostly pre-synaptically located α_2-adrenoceptors is one of inhibition, as their activation leads to decreased excretion of noradrenaline into the synaptic space (Langer, 1974; Berthelsen and Pettinger, 1977; Vargas and Gorman, 1995).

However, a significant subpopulation of peripheral α_2-adrenoceptors is involved, for instance, in regulating the vasomotor tone of vessels, thus influencing blood pressure (Piascik et al., 1996; Gyires et al., 2009). Genetic polymorphism both across and within these subpopulations has also been reported (Muskat et al., 2005; Kurnik et al., 2006). Thus, the net effect of any agonist or antagonist affecting the general population of α_2-adrenoceptors is characterized by a complex series of events within both the central nervous system (CNS) and the peripheral organ systems (Khan et al., 1999; Kamabayashi and Maze, 2000; Murrell and Hellebrekers, 2005).

2.2 α_2-adrenoceptors within the central nervous system

In the mammalian nervous system, the *nucleus locus coeruleus* (NLC) is an epicenter of noradrenergic pathways affecting both cortical activity and spinal modulation of selected afferent and efferent nerve fibers (Figure 1). A high prevalence of α_{2A}-adrenoceptors is found within the NLC, and they are thought to be responsible for mediating much of the sedative and antinociceptive effects of α_2-adrenoceptor agonists such as dexmedetomidine (Correa-Sales et al., 1992a; Scheinin and Schwinn, 1992). High densities of α_{2A}-adrenoceptors are also found in

the descending pathways within the spinal column, where they modulate nociceptive stimuli and interactions with opioids (Calzada and Artinano, 2001; Fairbanks et al., 2009). This subreceptor type is also likely to affect central control of vasomotor tone and is thought to mediate both hypotension and bradycardia in the presence of agonists, although the specific location of the α_2-adrenoceptors responsible for this effect remains unclear (Link et al., 1996; MacMillan et al., 1996; Nassar and Abdel-Rahman, 2006).

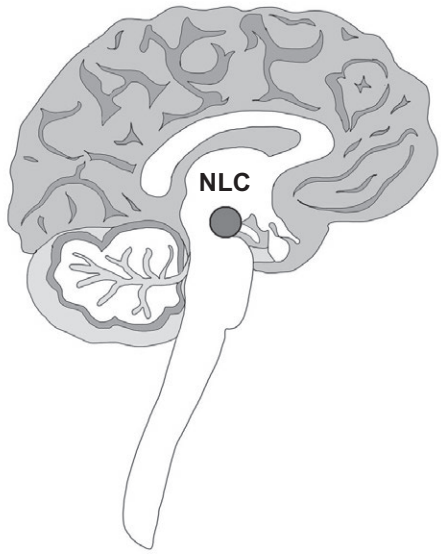


Figure 1. The nucleus locus coeruleus (NLC) within the mammalian CNS.

The α_{2C}-adrenoceptors, which are found in terminals of afferent primary sensory neurons, play a role in both mediating and modulating antinociception. This receptor subtype is involved in a synergistic interaction with opioids in producing analgesia at the spinal level (Fairbanks et al., 2009). The α_{2B}-adrenoceptors are scarce within the adult CNS and are limited in rats to the thalamic region (Scheinin et al., 1994), although they have been implicated in modulating N_20 mediated nociception in mice in concert with the α_{2A}-adrenoceptors (Guo et al., 1999; Sawamura et al., 2000).

2.3 Imidazoline receptors within the central nervous system

Some of the central hypotensive effects of α_2-adrenoceptor agonists have been attributed to non-adrenergic receptors located in the *nucleus reticularis lateralis* found in the ventrolateral medulla that specifically recognize an imidazoline-ring structure found, for instance, in dexmedetomidine and clonidine (Bousquet et al., 1984; Khan et al., 1999). Of the two isolated subtypes, I1 is mainly responsible for central control of blood pressure (Ernsberger et al., 1998a; Edwards et al., 2011). Imidazoline receptor agonists act as antihypertensives and are also thought to be more important than α_2-adrenoceptors in the central inhibition of catecholamine-induced dysrhythmias (Kamibayashi et al., 1995b; Mammoto et al., 1996). Imidazoline receptor agonists have not been shown to cause either sedation or antinociception (Khan et al., 1999; Prichard and Graham, 2000). In essence, central imidazoline receptors seem to have a complimentary effect with α_2-adrenoceptors.

2.4 Peripheral α_2-adrenoceptors

2.4.1 The α_{2B}-adrenoceptors

This subpopulation, which like other α_2-adrenoceptors is activated by both noradrenaline and adrenaline, mediates the typical vasoconstrictive effects induced by α_2-adrenoceptor agonists such as dexmedetomidine (Piascik et al., 1996; Link et al., 1996). This increase in arterial vasomotor tone is mediated via extrajunctional, postsynaptic receptors located in vascular smooth muscle, and the receptor-specific signal mechanism is linked with a voltage-gated Ca^{2+}-channel allowing the translocation of extracellular calcium (Ruffolo, 1985). As of late, the inhibition of ATP-sensitive potassium channels has been proposed as the underlying primary effector mechanism initiating the signaling cascade of dexmedetomidine-induced peripheral vasoconstriction in rat aortic cell preparations (Kawano et al., 2012). Nevertheless, the Ca^{2+}-channel antagonists nifedipine and isradipine normalized the increases in mean arterial pressure after dexmedetomidine administration in isoflurane-anesthetized dogs (Bloor et al., 1992a; Roekaerts et al., 1997). The α_{2B}-adrenoceptors are thought to preferentially mediate arterial rather than venous contraction in the presence of agonists (Link et al., 1996; Philipp and Hein, 2004).

2.4.2 The α_{2A}- and α_{2C}-adrenoceptors

The distribution and activity of vascular α_2-adrenoceptors varies between species, and their prevalence presumably differs based on the vascular type and location. In the dog, Polonia et al. (1985) postulated that while the predominant adrenoceptor population mediating vasoconstriction in canine arteries is type α_1, the net effect from α_2-adrenoceptors is more important within the venous vasculature. Further work by MacLennan et al. (1997) suggested that postsynaptic α_{2A}-adrenoceptors in the canine saphenous vein were largely responsible for the contractile effects of α-adrenoceptor agonists. Nimodipine, another Ca^{2+}-channel antagonist, inhibited α_2-adrenoceptor agonist-induced contraction of vascular smooth muscle in the canine saphenous vein model, while it had little effect on α_1-adrenoceptor agonists (Cooke et al., 1985).

Peripheral α_{2A}-adrenoceptors, as well as the α_{2C}-adrenoreceptors, have also been implicated in modulating hyperalgesia after experimental tissue injury (Fairbanks et al., 2002; Tomic et al., 2007). Furthermore, the α_{2A}-adrenoceptors have been suggested to enhance the peripheral action of local anesthetics (Yoshitomi et al., 2008). The role of α_{2C}-adrenoceptors in mediating arterial or venous vasoconstriction in dogs remains unclear.

2.5 Peripheral imidazoline receptors

The role of peripheral imidazoline receptors is controversial. Both I1 and I2 subtype receptors have been located within various organ systems, such as hepatic, renal, adrenal medullar, adipose and peripheral neuronal tissue (Ernsberger et al., 1998a; Khan et al., 1999). While within the CNS I1 receptors seem to be restricted to neuronal plasma membranes, in the periphery the majority of I2 receptors are found, for instance, within mitochondrial membranes (Regunathan et al., 1993) Furthermore, unlike the central receptors, the peripheral I2 subtype is not linked with a G-protein, and monoamine oxidation inhibition has been suggested as the primary signaling mechanism (Ernsberger et al., 1998a). However, these imidazoline receptors are not without pharmacological effect, and whilst difficult to completely distinguish from the actions of the adrenoceptors, it has been suggested that their

action is in fact distinct from, or often even opposite to, the peripheral α_2-adrenoceptors (Ernsberger at al., 1998b).

2.6 Dexmedetomidine

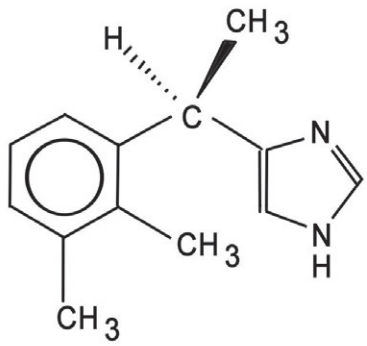


Figure 2. Dexmedetomidine. 5-[(1S)-1-(2,3-dimethylphenyl)ethyl]-1H-imidazole (IUPAC)

Dexmedetomidine, a selective and specific α_2-adrenoceptor agonist, is the dextroisomer of the racemic mixture, medetomidine (Aantaa et al., 1993; Kamibayashi and Maze, 2000). Medetomidine includes an equal concentration of levomedetomidine, the levoisomer thought to have little or no pharmacological activity in α_2-adrenoceptors (Kuusela et al., 2001a). Structurally, dexmedetomidine is a chiral methylol derivative including an imidazoline-ring and has a molecular weight of 200.3. Dexmedetomidine is currently licensed for sedation in dogs, cats and humans.

2.6.1 Cardiovascular effects of dexmedetomidine

The cardiovascular outcome of dexmedetomidine in dogs is well described, although not yet completely elucidated. It is commonly agreed that the hemodynamic effects of dexmedetomidine are a result of the activation of both central and peripheral α_2-adrenoceptors, as dexmedetomidine is readily distributed across the blood–brain barrier. In dogs, marked vasoconstriction and hypertension are initially seen, followed almost immediately by baroreflex-mediated bradycardia (Bloor et al., 1992a). Consequently, the cardiac index and oxygen delivery decrease as systemic vascular resistance and central venous pressure are increased (Flacke et al., 1993).

The following central sympatholysis and/or increase in parasympathetic tone leads to a reduction in systemic blood pressure as both the bradycardia and decrease in the cardiac index are sustained, suggestive of an inhibitory action on the baroreflex-mediated autonomic ventricular-vascular coupling (Xu et al., 1998). On the other hand, indirect suppression of the canine myocardium mediated through reduced catecholamine availability has also been suggested (Flacke et al., 1993; Roekaerts et al., 1997). A direct inhibitory effect on the contractility of the canine myocardium seems unlikely (Flacke et al., 1992). In denervated dogs, different administration regimens (intravenous bolus/infusions) lead to typical cardiovascular changes that were readily antagonized by atipamezole (Flacke et al., 1990).

Coronary vasoconstriction has been reported in dogs after dexmedetomidine, although its clinical significance remains unclear (Schmeling et al., 1991; Flacke et al., 1993). In fact, dexmedetomidine reduced hemodynamic indices of myocardial oxygen demand to a similar extent as recorded with esmolol, a β_1-adrenenoceptor antagonist, without actually reducing myocardial oxygen consumption (Willigers et al., 2004 and 2006). During experimentally induced myocardial ischemia in dogs, a dexmedetomidine infusion reduced the release of myocardial lactate when compared to saline, leading the authors to postulate that dexmedetomidine might have anti-ischemic properties in the canine myocardium (Willigers et al., 2003).

Acute α_2-adrenoceptor agonist-induced arrhythmias in dogs are characterized by the expected sinus bradycardia along with varying disturbances in sinoatrial and/or atrioventricular conductivity (Vainio and Palmu, 1989; Kramer et al., 1996). Kuusela et al. (2000 and 2001a) reported frequent 1st degree and occasional 2nd degree atrioventricular blocks accompanied by accentuated sinus arrhythmia and prolonged sinus pauses after both medetomidine and dexmedetomidine administration. Electrical abnormalities of ventricular origin have been infrequently reported. All changes in the electrocardiograms resolve spontaneously, and 24-hour Holter monitoring performed after medetomidine/dexmedetomidine sedation in healthy beagle dogs revealed no sustained arrhythmias (Kuusela et al., 2002). Dexmedetomidine has been shown to dose-dependently attenuate adrenaline-induced arrhythmias in halothane-anesthetized dogs, although this effect seems to be central in origin and more dependent on imidazoline receptors rather than α_2-adrenoceptors (Hayashi et al., 1993; Kamibayashi et al., 1995a and b). On the other

hand, clinically used doses of medetomidine, with or without atipamezole administration, failed to produce a similar effect (Pettifer et al., 1996).

In chloralose/urethane or halothane/fentanyl-anesthetized dogs, increasing doses of dexmedetomidine produced slightly different hemodynamic effects, while in both groups the distribution of the remaining cardiac output, measured 15 minutes after drug administration, was better preserved in more vital organs than, for instance, the skin or spleen (Lawrence et al., 1996). In a laser Doppler study, intestinal and skeletal, but not renal cortical microvascular perfusion was reduced by intramuscular medetomidine in dogs anesthetized with isoflurane (Pypendop and Verstegen, 2000). Premedication with intramuscular medetomidine increased the CNS uptake of a lipophilic tracer during brain perfusion imaging in healthy dogs, leading the authors to postulate that the central distribution of the tracer might have been enhanced as a consequence of a lower proportionate decrease in CNS perfusion when compared to peripheral tissues (Waelbers et al., 2011). Moreover, intravenous dexmedetomidine reduced cerebral oxygen transport during normoxia, but did not impair a hypoxia-induced increase in cerebral blood flow in isoflurane-anesthetized dogs (McPherson et al., 1994). Although the central and peripheral effects on cardiovascular function have been difficult to distinguish, the peripheral hemodynamic effects seem to be accomplished with lower doses than the central ones in canines, with higher doses leading to the prolongation of both effects (Pypendop and Verstegen, 1998; Kuusela et al., 2000).

2.6.2 Respiratory effects of dexmedetomidine

A decreased respiratory rate is the most commonly reported finding with either medetomidine or dexmedetomidine in dogs, along with mild reductions in arterial oxygen tension (Vainio, 1989; Kramer et al., 1996; Ko et al., 1996; Kuusela et al., 2000). Arterial carbon dioxide concentrations remain unaffected, although a reduced ventilatory response during hypercapnia has been reported for dexmedetomidine (Sabbe et al., 1994). Equipotent doses of medetomidine and dexmedetomidine produced similar alterations in arterial blood gas tensions (Kuusela et al., 2001a). However, when combined with opioids, significant hypoxemia and hypercapnia can occur (Ko et al., 1996; Raekallio et al., 2009). Interestingly, high doses of dexmedetomidine have also been described to increase minute ventilation in dogs,

an effect that was abolished by concurrent administration of isoflurane (Nguyen et al., 1992).

2.6.3 Central effects of dexmedetomidine

Dexmedetomidine induces a dose-dependent degree of sedation by inhibiting monoaminergic transmission (i.e. noradrenaline, serotonin and dopamine) within the CNS (Rabin et al., 1996; Millan et al., 2000; Lähdesmäki et al., 2003). A diversity of additional or contributing mechanisms, such as increased GABAergic activity, decreased glutamanergic neurotransmission and alterations in cerebellar cGMP activity, have also been suggested to participate in dexmedetomidine's central mechanisms of action (Seidel et al., 1995; Vulliemoz et al., 1996; Huang and Hertz, 2000; Chiu et al., 2011). While the specific effector mechanism varies between different α_2-adrenoceptor subtypes and/or locations, cellular effects are produced via a G-protein-linked signaling mechanism (Kamibayashi and Maze, 2000). More specifically, dexmedetomidine yields its hypnotic action by altering the degree of phosphorylation of potassium channels via inhibition of the action of adenylate cyclase, thus hyperpolarizing the neuronal plasma membrane (Correa-Sales et al., 1992b; Shirasaka et al., 2007). The consequent inhibition of the voltage-dependent Ca^{2+} channels is likely to be linked to the ultimate suppression of neurotransmitter release into the synaptic spaces (Hayashi and Maze, 1993; Chiu et al., 2011).

The main central antinociceptive action of dexmedetomidine is thought to be mediated via spinal α_2-adrenoceptors located in the *substantia gelatinosa* within the dorsal horns of the spinal cord (Howe et al., 1983; Kuraishi et al., 1985; Hayashi et al., 1995). However, a supraspinal component via the NLC has also been suggested (Pertovaara et al., 1991; Guo et al., 1996). In fact, α_2-adrenoceptor agonists have been reported to produce an analgesic action when administered parenterally, epidurally or intrathecally (Khan et al., 1999; Kamibayashi and Maze, 2000).

In dogs, both medetomidine and dexmedetomidine produce a dose-dependent degree of sedation after intravenous or intramuscular administration (Vainio, 1989; Sabbe et al., 1994). Furthermore, higher doses will prolong the sedative effects, while the intensity of the sedation is not increased further after a ceiling effect (Pypendop and Verstegen 1998; Kuusela et al., 2000). Studies on the analgesic effects of medetomidine and dexmedetomidine have yielded varying results in dogs,

probably due to the difficulties in differentiating between sedation and analgesia (i.e. perception and/or inhibition of a nociceptive pathway) within various experimental settings. Interestingly, van Oostrom et al. (2010) suggested that higher plasma concentrations of dexmedetomidine are necessary to attenuate a somatosensory than an auditory response in dogs.

2.6.4. Other effects of dexmedetomidine

Due to the wide distribution of different α_2-adrenoceptors throughout the mammalian body, all relevant α_2-adrenoceptor agonists produce a variety of pharmacological effects on different organ systems. One of the most relevant effects is pronounced diuresis, which is thought to be caused by inhibition of the antidiuretic hormone (Jackson et al., 1992; Saleh et al., 2005; Villela et al., 2005). The diuretic effect can be antagonized with atipamezole (Talukder et al., 2009). Medetomidine and dexmedetomidine have also been implicated in interfering with blood glucose homeostasis via insulin inhibition, an effect suggested to be mediated via the α_{2A}-adrenoceptors (Burton et al., 1997; Fagerholm et al., 2004).

Medetomidine and dexmedetomidine have also been shown to modulate physiological stress responses in dogs (Ko et al., 2000; Kuusela et al., 2003). Compared to saline, premedication with medetomidine blunted the elevations in blood adrenaline, noradrenaline, adrenocorticotropic hormone and insulin concentrations during ovariohysterectomy in dogs (Benson et al., 2000). Furthermore, blood catecholamine, but not beta-endorphin concentrations, were significantly lower with medetomidine than with acepromazine premedication during a similar study setting (Väisänen et al., 2002). In humans, dexmedetomidine has also been reported to decrease the temperature threshold of shivering (Talke et al., 1997; Weant et al, 2010; Bajwa et al., 2012).

2.6.5 Pharmacokinetics of dexmedetomidine

The hemodynamic effects of dexmedetomidine have been suspected to influence its pharmacokinetic behavior (Salonen et al., 1995; Kuusela et al., 2000; Escobar et al., 2012). Kuusela et al. (2000) separately examined the pharmacokinetics of both dexmedetomidine and levomedetomidine in dogs. After single IV doses of 10 and 20

µg/kg, steady-state Vd for dexmedetomidine remained below 1 L/kg compared to respective values above 2.5 L/kg for the levoisomer. Similarly, the clearance of dexmedetomidine was less than a third of that for levomedetomidine as the marked decrease in the cardiac output could logically influence plasma concentrations of the active dextroisomer (Dutta et al., 2000; Pypendop et al., 2012). Salonen et al. (1995) showed that administration of atipamezole increased the clearance of medetomidine in dogs, leading the authors to postulate that metabolism of the latter was influenced by changes in hepatic blood flow. On the other hand hepatic biotransformation, rather than the degree of liver perfusion, has been suggested as the rate-limiting step in the metabolic clearance of racemic medetomidine (Salonen et al., 1989). In two *in vitro* studies, a low rate of biotransformation by canine hepatocytes has been described for both medetomidine and dexmedetomidine (Kaivosaari et al., 2000; Duhamel et al., 2010). Overall, while some of these discrepancies in the pharmacokinetic behavior between the two enantiomers could be explained by differences in rates of their hepatic biotransformation, equipotent doses of racemic medetomidine and dexmedetomidine still produced comparably behaving plasma concentrations in dogs (Kuusela et al., 2000). The hepatic extraction ratio of dexmedetomidine has not been reported for dogs.

2.7 MK-467

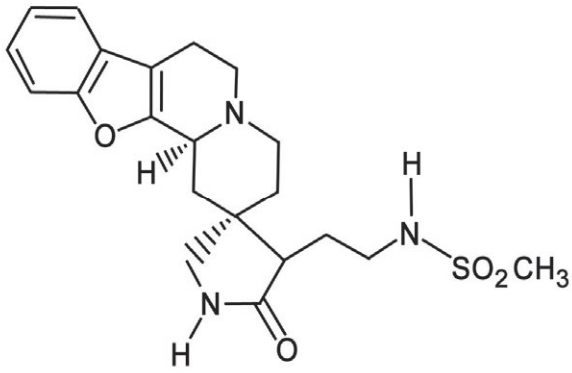


Figure 3. MK-467. N-[2-[(2R,12bS)-2'-oxospiro[1,3,4,6,7,12b-hexahydro-[1]benzofuro[2, 3-a]quinolizine-2,5'-imidazolidine]-1'-yl]ethyl]methanesulfonamide (IUPAC)

MK-467 was first introduced as L-659,066 by Clineshcmidt et al. (1988) as a novel, peripherally active α_2-adrenoceptor antagonist. Structurally, MK-467 is a spirocyclic-substituted benzofuroquinolizine without an imidazoline-ring structure and has a molecular weight of 418.7. In their pioneer study, the researchers showed that systemically administered MK-467 did not reverse clonidine-induced mydriasis in rats, while it attenuated an increase in diastolic blood pressure by UK-14,304, a selective α_2-adrenoceptor agonist. The potency of MK-467 in antagonizing the increase in withdrawal times of UK-14,304-pretreated mice exposed to a hot-plate proved low. The authors also studied the distribution of systemically administered radiolabelled MK-467 into the central nervous system of rats and marmosets, finding that the brain:plasma ratios for both total radioactivity and the free drug concentrations were approximately 1:20. As for α_2:α_1 receptor selectivity, both *in vivo* and *in vitro* studies demonstrated a substantial preference for the α_2-adrenoceptor, with ratios ranging from 30–100:1 depending on the study setting (Clineschmidt et al., 1988). Later, MK-467 was reported not to affect dexmedetomidine-induced sedation in rats (Doze et al., 1989).

2.7.1 Cardiovascular effects of MK-467

MK-467 was shown to increase heart rates in concert with a slight decrease in mean arterial pressure in conscious rats, an effect that was suggested to be due to increased noradrenaline release due to a decrease in vasomotor tone induced via peripheral α_2-adrenoceptor antagonism (Szemeredi et al., 1989). In healthy human volunteers, MK-467 caused a small increase in systolic blood pressure within one hour of peroral administration (Warren et al., 1991). Intravenously administered MK-467 did not alter supine or erect systolic blood pressures in humans, although a slight increase in heart rates was observed when compared to placebo (Schafers et al., 1992). Moreover, MK-467 did not significantly accentuate or attenuate exercise-induced increases in heart rate and arterial blood pressure in healthy volunteers (Sciberras et al., 1994).

In dogs, MK-467 administered as an intravenous infusion dose-dependently increased heart rates and cardiac output while reducing systemic vascular resistance when measured 30 minutes after drug administration. No significant effects on mean arterial pressures were observed (Pagel et al., 1998). Enouri et al. (2008) reported

similar findings five minutes after rapid intravenous administration of a single dose of MK-467 in dogs.

2.7.2 Other effects of MK-467

MK-467 reduced the urine output in normally hydrated rats, but had little effect on rats that were void of the antidiuretic hormone (ADH), leading the authors to suggest that α_2-adrenoceptor agonists inhibit the action of ADH in the collecting tubules (Jackson et al., 1992). In healthy human volunteers, no effects on resting blood glucose or insulin levels were detected after treatment with MK-467, although an increased response in insulin and free fatty acid levels was observed after exercise (Schafers et al., 1992; Sciberras et al., 1994). Both of these studies in humans confirmed an increase in plasma noradrenaline concentrations after administration of MK-467.

2.7.3 Pharmacokinetics of MK-467

No published reports on the pharmacokinetics of MK-467 in any species were located. In humans, plasma concentrations ranging from 165–890 ng/mL have been reported after differing dosing regimens (Schafers et al., 1992; Sciberras et al., 1994).

2.8 Atipamezole

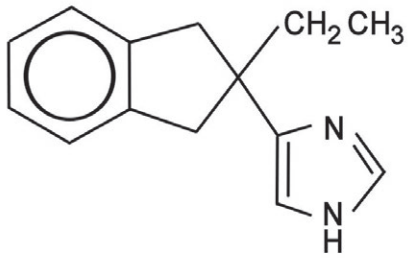


Figure 4. Atipamezole. 5-(2-ethyl-1,3-dihydroinden-2-yl)-1H-imidazole (IUPAC)

Atipamezole is a specific α_2-adrenoceptor antagonist, with an α_2:α_1 selectivity ratio of 8500:1. Atipamezole reverses the central and peripheral effects of both medetomidine and dexmedetomidine in dogs (Vähä-Vahe, 1990; Vainio, 1990; Flacke et al., 1990). Atipamezole contains an imidazoline-ring and has a molecular weight of 212.3. There are no published reports on the cardiovascular or central function when administered alone in dogs. In healthy human volunteers, increasing plasma concentrations of atipamezole induced moderate elevations in diastolic and systolic arterial pressures, with negligible effects on overall heart rates (Penttilä et al., 2004).

2.9 The use of antimuscarinic drugs with α_2-adrenoceptor agonists

Antimuscarinics, such as atropine and glycopyrrolate, exhibit their action via muscarinic receptors expressed mainly in the sinoatrial, atrioventricular and atrial myocardium in the canine heart (Kent et al., 1974). Both drugs act as vagolytics, thus enhancing sinoatrial automacity and atrioventricular conduction. They have been used to counteract vagally mediated bradycardia, as they increase the heart rate and blood pressure (Plunkett and McMichael, 2008). Consequently, they have been studied in either the prevention or correction of bradycardia induced by α_2-adrenoceptor agonists in dogs. Short (1991) described an elevated incidence of cardiac dysrhythmias when atropine or glycopyrrolate was administered after medetomidine in dogs, and reported similar, whilst less severe, outcomes when the two antimuscarinics were given pre-emptively. Alibhai et al. (1996) noted prolonged hypertension in a similar study setting. Intramuscular administration of increasing doses of medetomidine in atropine-preteated (IM) dogs led to severe hypertension, irrespective of the dose administered, when compared to medetomidine alone (Ko et al., 2001). Furthermore, Alvaides et al. (2008) reported pronounced hypertension induced by dexmedetomidine in atropine-premedicated dogs, an effect that was only slightly attenuated by acepromazine, an α_1-adrenoceptor antagonist tranquilizer.

Importantly, Bloor et al. (1992a) had already demonstrated that while pre-treatment with glycopyrrolate did prevent the reduction in heart rates induced by dexmedetomidine, the cardiac index was only moderately improved and returned to baseline levels only after reversal with atipamezole. More recently, Congdon et al. (2011) confirmed a similar decrease in cardiac output after both dexmedetomidine

(IM) and dexmedetomidine with atropine (IM) in conscious dogs. In this study, ventricular arrhythmias (ventricular premature contractions and ventricular bigemini) were also observed when dexmedetomidine was given with the antimuscarinic drug.

2.10 Current knowledge of concomitant use of dexmedetomidine and MK-467

In a pioneering study, Doze et al. (1989) found that MK-467 was unable to antagonize the hypnotic action of dexmedetomidine in rats, while idaxozan, a central and peripheral α_2-adrenoceptor antagonist, completely abolished its central effects. No studies on the effects of MK-467 on medetomidine or dexmedetomidine-induced sedation in dogs were located.

In a detailed investigation, Pagel et al. (1998) demonstrated that MK-467 was able to dose-dependently attenuate the cardiovascular effects of dexmedetomidine in dogs. The authors used infusion regimens to administer increasing doses of MK-467 (0.1, 0.2 and 0.4 mg/kg IV) prior to a 5 µg/kg dose of dexmedetomidine with a 24 h wash-out period between treatments. Measurements were made at baseline, 30 minutes after MK-467 and 5 and 60 minutes after dexmedetomidine. They reported a typical early hemodynamic outcome after dexmedetomidine alone, albeit only a moderate early decrease in heart rate. In contrast, MK-467 induced a reduction in systemic vascular resistance coupled with an increase in the heart rate and cardiac output, resulting in stable mean arterial pressures. After MK-467, dexmedetomidine was able to reduce heart rates with all but the highest MK-467 dose. Surprisingly, the bradycardic effect was less pronounced with dexmedetomidine alone. However, as afterload was decreased with higher doses of MK-467, cardiac output was better maintained in the presence of the antagonist. The authors postulated that while MK-467 accentuated the later central sympatholytic effects of dexmedetomidine, as described by more pronounced reductions in mean arterial and left ventricular systolic pressures, the overall outcome was balanced out by the peripheral post-synaptic α_2-adrenoceptor antagonism.

Similarly, premedication with 0.2 mg/kg of MK-467 IV attenuated the cardiopulmonary effects of 10 µg/kg of medetomidine in dogs (Enouri et al., 2008). Again, medetomidine administration led to a decrease in the heart rate and cardiac index, but the effects were significantly less in the presence of the antagonist. MK-467 had no effect on respiratory rates or arterial blood gases when compared to

saline premedication, although the antagonist attenuated the increase in medetomidine-induced tissue oxygen extraction ratio, proving the significance of the reduction in tissue oxygen delivery in the absence of the antagonist. Furthermore, combining glycopyrrolate (5 µg/kg) with MK-467 showed no further hemodynamic improvement or a significant increase in oxygen delivery.

Earlier, Hayashi et al. (1991) noted that while MK-467 did not affect the antiarrhythmic effect of dexmedetomidine when halothane-anesthetized dogs were administered adrenaline, it reduced blood pressures and increased heart rates compared to dexmedetomidine alone. This effect was not seen when MK-467 was administered cerebroventricularly in a similar study setting (Kamibayashi et al., 1995b).

In horses and sheep pretreated with methoxamine (an α_1-adrenoceptor agonist), MK-467 attenuated medetomidine-induced hypertension and bradycardia (Bryant et al., 1998). Furthermore, simultaneous administration of 250 µg/kg of MK-467 abolished all cardiovascular effects of 5 µg/kg of dexmedetomidine in conscious sheep (Raekallio et al., 2010).

Recently, Restitutti et al. (2012) demonstrated that MK-467 blunted both the decrease in plasma insulin and the increase in glucose concentrations induced by dexmedetomidine in dogs. These findings are consistent with previous reports on the effects of MK-467 on clonidine-induced changes in plasma insulin and glucose levels in humans (Warren et al., 1991).

MK-467 has also been shown to prevent the analgesic action of dexmedetomidine in rats with peripherally induced neuropathy, leading the authors to suggest that peripheral α_2-adrenoceptors may play a role in the modulation of neuropathic pain outside the central nervous system (Poree et al., 1998). Elsewhere, MK-467 had no effect on the antinociceptive effects of dexmedetomidine in acute visceral pain induced by colorectal distension (Ulger et al., 2009).

3. Aims of the study

1) To investigate the effects of various doses of MK-467 on the cardiopulmonary action of dexmedetomidine, with special reference to simultaneous administration.
2) To characterize the effects of MK-467 and dexmedetomidine on their plasma concentrations and describe their clinical consequences.
3) To define any short-term clinical or cardiopulmonary adverse effects of MK-467 when administered alone.
4) To evaluate the clinical quality of dexmedetomidine-induced sedation in the presence of MK-467 and its reversal with atipamezole

4. Material and methods

4.1 Animals and instrumentation

In Study I, six healthy neutered laboratory beagles, three males and three females, were used. They were aged between 9 and 11 years and no invasive monitoring techniques were applied.

For the rest of the studies (II–IV), eight healthy laboratory beagles, six males and two females, were used. The dogs were neutered six months prior to the first experiments and adapted to both handling and the research environment. These dogs were aged 15 (2.3) months (mean (SD)) during the experiments.

All dogs were routinely vaccinated and dewormed, and they were housed in groups with daily access to outdoor exercise and fed a commercial diet. The health status of the dogs was assessed by thorough clinical examinations, complete blood counts and routine serum chemistry, repeated periodically and when thought necessary.

In study I, a venous catheter was placed into the cephalic vein of the dogs and no other invasive instrumentation was applied. For studies II–IV, the arterial and central venous catheterizations were performed under sevoflurane anesthesia and after extubation, the dogs were allowed to recover for a minimum of one hour prior to baseline measurements. All studies were approved by the National Animal Experimentation Board.

4.2 Drugs and dosages

A summary of the performed studies is presented in Table 1. Dexmedetomidine (Dexdomitor, Orion Pharma, Turku, Finland), atipamezole (Antisedan, Orion Pharma) and MK-467 (Merck, Sharpe&Dohme, PA, USA) were used in the studies. Commercial preparations of dexmedetomidine and atipamezole were used and diluted where appropriate, whereas MK-467 was supplied as a powder and reconstituted with saline in a sterile vial to form a clear, homogeneous injectable solution (1 mg/mL) prior to each treatment. All IV drug treatments were drawn into a single syringe and further diluted with saline to an equal final volume of 10 mL. In study I, atipamezole was given undiluted.

In study I, 5 µg/kg of dexmedetomidine with or without 250 µg/kg of MK-467 was administered IV to each dog on two separate occasions with a minimum seven-day wash-out period between treatments. Atipamezole (50 µg/kg) was administered IM 40 minutes after both treatments.

Table 1. Summary of study protocols.

Study	Number of animals	Treatments (µg/kg, IV)	Primary outcome	Secondary outcome(s)
I	6	a. D 5 b. D 5 + MK 250 ATI 50 (IM)	Clinical sedation	Pulse rate, atipamezole reversal
II	8	a. D 10 b. D 10 + MK 250 c. D 10 + MK 500 d. D 10 + MK 750 e. MK 250	Cardiorespiratory parameters	
III	8		Clinical sedation	
IV	8 (5)		Plasma concentrations	

D = dexmedetomidine, MK = MK-467, ATI = atipamezole

In studies II–IV, 10 µg/kg of IV dexmedetomidine was given alone or in combination with 250, 500 or 750 µg/kg of MK-467. Additionally MK-467 (250 µg/kg) was also given alone. Each dog was hence studied five times and a minimum wash-out period of 14 days was allowed between treatments. Intravenous treatments were administered over a 30–second period in all studies.

4.3 Cardiorespiratory monitoring

In study I, pulse rates were assessed by manual femoral pulse wave assessments during 30 seconds. In an effort to minimize monitoring-induced alterations in the level of sedation, no other cardiovascular methods were applied after drug administration. On a separate occasion, a light (150 g), portable Holter-monitoring device designed by Hyvärinen et al. (2006) was also used in order to obtain minimum resting heart rates for comparison with treatment-induced pulse rates. The monitor was integrated in a textile vest that all dogs became accustomed to wearing prior to the recordings.

In study II, a modular multiparameter monitor (S/5 Compact Critical Care Monitor, Datex-Ohmeda, Hatfield, UK) was used to record a continuous lead II electrocardiogram as well as invasive arterial and central venous pressures. Arterial catheters were placed into the metatarsal artery and the double lumen central venous catheters were introduced via the jugular vein. Both invasive pressure transducers were calibrated prior to baseline measurements (X-Caliber®, Datex-Ohmeda).

Samples for arterial blood gas analysis were taken via the metatarsal arterial catheter into pre-heparinized syringes, stored in iced water and analyzed within 90 minutes. The arterial hemoglobin content, pH, PaO_2, $PaCO_2$, bicarbonate (HCO_3^-), electrolyte and lactate concentrations were recorded using a Radiometer ABL-800 Flex (Radiometer, Copenhagen, Denmark) analyzer. As the dogs were breathing room air during study II, $P(A\text{-}a)O_2$ was also provided by the analyzer. Respiratory rates were calculated by observing chest wall movements.

Cardiac output measurements were performed with the lithium dilution method (LidCO™Plus Hemodynamic Monitor, LidCO Ltd, Cambridge, UK), as previously described in dogs (Mason et al., 2001). The LidCO computer requires blood hemoglobin and sodium concentrations to produce accurate CO values, and standard concentrations of 10 g/dL and 140 mmol/L were used for hemoglobin and sodium, respectively. As these values were simultaneously acquired with the blood gas analyzer but analyzed with a delay, LidCo Ltd provided the investigators with a formula that allowed the correction of CO values, integrating the actual concentrations for both required parameters. The PulseCo feature of the monitor, which yields a continuous CO estimate derived from the arterial pulse waveform, was not used during the experiments.

The following formulae were used to calculate the derived parameters in study II:

- Cardiac index (mL/kg/min) = cardiac output / body weight (Boyd et al., 1991)
- Systemic vascular resistance (dynes x s x cm^{-5}) = 80 x (MAP-CVP) / CO (Boyd et al., 1991);
- Arterial hemoglobin saturation (%) = $[37\,900/(PaO_2^3 + 205 \times PaO_2) + 1]^{-1}$ (Reeves et al., 1982);
- Arterial blood oxygen content (mL/mL) = $[(1.39 \times Hb \times S_aO_2) + (0.0031 \times PaO_2)] \times 0.01$ (Boyd et al., 1991);

• Oxygen delivery (mL/kg/min) = Cardiac index x Arterial blood oxygen content (Boyd et al., 1991);

• Alveolar-to-arterial oxygen partial pressure difference = {0.21 x ($pressure_{ambient}$ – 6.275) – pCO_2 x [RQ^{-1} – 0.21 x (RQ^{-1} – 1)] } – PaO_2 , where RQ (respiratory quotient) is 0.86 by default (Radiometer ABL800 Flex Reference Manual).

4.4 Plasma concentrations of dexmedetomidine

Blood samples for analysis of plasma dexmedetomidine concentrations were taken in study IV. Samples were obtained via a designated port of the central venous catheter, centrifuged and stored at -20 °C until analyzed with liquid chromatography-mass spectrometry as previously described (Snapir et al., 2006). Standard software (WinNonlin Professional software package, version 5.2, PharSight Corporation, Mountain View, CA, USA) was used to calculate common pharmacokinetic parameters using non-compartmental methods. Half-lives for the distribution and elimination phases were calculated with a two-compartment IV bolus model with no lag time and first-order elimination.

4.5 Plasma concentrations of MK-467

MK-467 concentrations in plasma from five dogs in study IV were analyzed with liquid chromatography–mass spectrometry after liquid-liquid extraction and with yohimbine as an internal standard. After reversed-phase separation (Gemini 5μ C_{18} 110A, 150 x 2.0 mm, Phenomenex), quantitative detection was performed in a multi-reaction monitoring mode (MRM) with a triple quadrupole mass spectrometer (AB Sciex API 4000). For MK-467 and yohimbine, the precursor ions were scanned at m/z of 419.0 and 355.0, respectively. The fragment ions monitored for MK-467 had m/z values of 127.0 and 200.0 and those for yohimbine were 212.0 and 144.0. The chromatograms were analyzed and processed using AB Sciex software (Analyst 4.1). Pharmacokinetic parameters were calculated with non-compartmental methods as described above for dexmedetomidine.

4.6 Clinical assessment of sedation

Sedation was subjectively assessed in studies I and III by an investigator blinded to the treatments. In study I, a composite sedation score designed by Kuusela et al. (2000) was applied. The same scoring system was used in study III with minor modifications (i.e. omitting the significance of spontaneous recumbency, as the dogs were gently held in a lateral position when necessary, regardless of the treatment). In study I, sedation scoring was continued for 20 minutes after atipamezole administration. In study III, sedation assessments were made up until 90 minutes after drug administration, with the exception of MK-467 alone, where the experiment was discontinued after 60 minutes. The area under the time-sedation curve was calculated by a trapezoidal method.

4.7 Methods for analgesia during the experiments

Due to repeated catheterizations, local infiltration of 5 mg of lidocaine (Lidocain® 20 mg/mL, Orion Pharma) was applied for each catheter insertion site, and 0.2 mg/kg of meloxicam (Metacam 5 mg/mL, Boehringer Ingelheim Vetmedica GmbH, Ingelheim, Germany) was administered intravenously at the end of each experiment in studies II–IV.

4.8 Study designs

Dexmedetomidine was chosen as the positive control in all studies, and no negative controls were used. In study I, the treatments were randomly allocated in a cross-over design and the investigator assessing sedation was blinded to the treatment. In studies II–IV, where five different treatments were investigated, a similar random allocation was applied, and the investigator monitoring sedation in study III was blinded to the treatment.

4.9 Statistical analysis

Repeated-measures analysis of variance was applied for numerical variables throughout the studies, followed by a *post-hoc* correction between treatments and

against baseline measurements where applicable. The Bonferroni *post hoc* correction was used for all comparisons except for the pulse/heart rates in study I, where the Dunnett's correction was used (minimum resting heart rate as the control). Shapiro-Wilk's test was used to evaluate whether the data followed a normal distribution. Bioequivalence was assessed as recommended by the European Medicines Agency (2001) in studies I and III. Data are expressed as the mean (CI95) and/or mean (SD). Values of $p < 0.05$ were considered significant. Data for studies II–IV were collected during the same series.

5. Results

5.1 Cardiovascular effects

5.1.1 Cardiovascular effects of dexmedetomidine (I, II)

Intravenous dexmedetomidine administration (10 µg/kg) in study II was followed by a rapid increase in systolic, mean, diastolic and central venous blood pressures, accompanied by a marked reduction in heart rate and cardiac index (Table 2). Consequently, systemic vascular resistance considerably increased and oxygen delivery was significantly reduced. All of these effects were most pronounced five minutes after dexmedetomidine administration. However, despite the following gradual reductions in systemic blood pressures after the initial hypertension, heart rates, cardiac index and oxygen delivery remained significantly below baseline levels throughout the 90-minute period. Moreover, systemic vascular resistance remained elevated when compared to the baseline. In accordance, 5 µg/kg of dexmedetomidine significantly reduced pulse rates from 5 to 40 minutes in study I. Atipamezole increased pulse rates within 10 minutes of IM administration, although baseline levels were not reached within the follow-up period.

Accentuated sinus bradyarrhythmia was the most common ECG abnormality observed after dexmedetomidine administration in study II. During the early observational period, and especially during the first five minutes, second-degree atrio-ventricular blocks were occasionally seen in all but one dog. First-degree atrio-ventricular blocks and prolonged sinus pauses were detected in all dogs. In two dogs, a single ventricular escape beat was detected between 10 and 20 minutes after administering dexmedetomidine, and in one dog, a total of four individual ventricular escape beats were seen within 10 minutes of drug administration.

5.1.2 Cardiovascular effects of MK-467 (II)

In study II, 250 µg/kg of MK-467 IV was followed by a rapid increase in the heart rate, cardiac index and oxygen delivery with no apparent changes in arterial or central venous pressures and a moderate but statistically significant decrease in systemic vascular resistance (Table 2). The median heart rate five minutes after MK-467 was 143/min, and the highest individual heart rate recorded was 208/min. All parameters started to return towards the baseline ten minutes after drug administration, and while both the cardiac index and oxygen delivery remained slightly elevated accompanied by the decrease in systemic vascular resistance, no statistically significant differences were found after 60 minutes when compared to the baseline. Besides the transient sinus tachycardia, no further ECG abnormalities were detected after MK-467 administration.

5.1.3 Cardiovascular effects of dexmedetomidine combined with MK-467 (I, II)

In study I, simultaneous administration of 250 µg/kg of MK-467 with 5 µg/kg of dexmedetomidine attenuated the bradycardia. Although pulse rates did decrease significantly, they remained above 60 bpm and were assessed as bioequivalent with the minimum resting heart rates acquired from the overnight Holter monitoring. Atipamezole increased pulse rates within 10 minutes, but similarly to dexmedetomidine alone, baseline levels were not reached.

In study II, MK-467 had a dose-dependent effect on the hemodynamic alterations induced by dexmedetomidine (Table 2). With the lowest dose (250 µg/kg), some early effects attributable to dexmedetomidine (bradycardia, increased central venous pressure, decreased cardiac index and oxygen delivery) were still evident. While no early hypertension was observed, mean arterial blood pressures decreased significantly after 20 minutes and remained below the baseline until 90 minutes. The lowest mean arterial pressures were recorded between 45 and 60 minutes after drug administration, with one dog having a single value below 60 mmHg (59 mmHg). Systemic vascular resistance, the cardiac index and oxygen delivery did not differ significantly from baseline values after five minutes. First-degree atrio-ventricular blocks were detected in all and second degree atrio-ventricular blocks were also seen in four dogs during the first five minutes.

Table 2. Hemodynamic parameters from study II. Results are expressed as mean (CI95). NM = not measured.

Parameter	Treatment	Baseline	5 min	10 min	20 min	30 min	45 min	60 min	75 min	90 min
HR (1/min)	D	96 (9)	28 (3)	33 (2)	36 (4)	38 (3)	43 (4)	50 (6)	53 (2)	55 (3)
	DM250	90 (9)	65 (8)	70 (7)	76 (9)	76 (7)	73 (6)	73 (6)	71 (9)	71 (11)
	DM500	94 (8)	81 (8)	87 (8)	81 (10)	76 (7)	71 (5)	71 (6)	67 (4)	66 (7)
	DM750	91 (10)	96 (13)	100 (12)	90 (9)	84 (7)	77 (9)	75 (8)	80 (11)	78 (15)
	M250	103 (9)	143 (25)	132 (25)	128 (20)	122 (18)	110 (14)	93 (13)	NM	NM
SAP (mmHg)	D	182 (4)	227 (15)	200 (11)	181 (9)	168 (9)	162 (9)	158 (10)	155 (8)	157 (11)
	DM250	185 (6)	181 (20)	160 (16)	146 (16)	138 (15)	136 (15)	136 (14)	144 (16)	138 (12)
	DM500	173 (7)	158 (17)	145 (18)	130 (16)	127 (15)	128 (12)	130 (13)	130 (9)	144 (10)
	DM750	181 (9)	146 (16)	120 (21)	118 (15)	119 (13)	121 (8)	124 (11)	142 (16)	156 (12)
	M250	184 (14)	197 (13)	207 (19)	195 (18)	190 (19)	197 (3)	180 (15)	NM	NM
MAP (mmHg)	D	108 (3)	166 (6)	146 (13)	127 (12)	116 (9)	108 (10)	102 (9)	98 (7)	98 (8)
	DM250	108 (5)	110 (9)	98 (10)	88 (10)	82 (9)	79 (9)	80 (10)	81 (9)	81 (8)
	DM500	106 (7)	97 (9)	86 (10)	78 (9)	73 (6)	74 (6)	76 (6)	75 (8)	83 (7)
	DM750	105 (6)	87 (10)	73 (10)	70 (6)	69 (6)	69 (4)	71 (6)	84 (11)	90 (8)
	M250	104 (9)	109 (11)	110 (14)	103 (10)	103 (10)	105 (10)	100 (10)	NM	NM
DAP (mmHg)	D	81 (4)	140 (8)	123 (14)	106 (8)	97 (9)	88 (9)	82 (8)	79 (7)	77 (9)
	DM250	82 (5)	86 (8)	76 (10)	69 (9)	63 (8)	62 (8)	62 (9)	62 (7)	63 (7)
	DM500	80 (6)	73 (7)	65 (8)	60 (7)	56 (6)	57 (5)	59 (5)	57 (7)	63 (7)
	DM750	79 (5)	67 (9)	55 (9)	53 (5)	53 (5)	53 (4)	56 (5)	66 (11)	70 (7)
	M250	76 (8)	80 (11)	81 (14)	73 (9)	74 (8)	75 (10)	73 (10)	NM	NM
CI (mL/kg/min)	D	171 (35)	51 (11)	57 (10)	59 (12)	61 (12)	64 (13)	73 (14)	82 (16)	85 (16)
	DM250	158 (11)	109 (15)	121 (18)	142 (30)	150 (18)	136 (12)	139 (17)	135 (25)	146 (17)
	DM500	156 (27)	151 (26)	164 (43)	171 (43)	143 (28)	145 (29)	127 (17)	120 (17)	174 (49)
	DM750	167 (30)	178 (29)	187 (48)	163 (33)	161 (24)	144 (25)	151 (33)	165 (50)	220 (56)
	M250	206 (36)	285 (53)	297 (66)	298 (77)	281 (66)	296 (70)	260 (60)	NM	NM
SVR ($dyn*sec/cm^5$)	D	3496 (627)	17577 (3380)	13362 (2538)	11843 (3531)	10198 (2280)	8830 (1919)	7523 (1752)	6411 (1330)	6100 (1154)
	DM250	3649 (642)	5236 (915)	4322 (872)	3393 (732)	2880 (430)	3060 (472)	3083 (572)	3270 (552)	3197 (252)
	DM500	3720 (589)	3483 (610)	3181 (1035)	2655 (642)	2835 (549)	2863 (491)	3152 (313)	3378 (298)	2794 (509)
	DM750	3309 (373)	2539 (377)	2139 (556)	2309 (394)	2236 (339)	2462 (334)	2491 (340)	2842 (353)	2219 (313)
	M250	2584 (434)	2002 (377)	2000 (419)	1923 (482)	2036 (532)	1908 (356)	2096 (523)	NM	NM
DO_2 (mL/kg/min)	D	34 (7)	10 (2)	11 (2)	11 (2)	12 (3)	12 (3)	15 (3)	16 (3)	17 (3)
	DM250	33 (2)	20 (3)	23 (4)	28 (7)	29 (4)	27 (3)	28 (4)	32 (4)	32 (3)
	DM500	30 (5)	27 (4)	28 (6)	32 (9)	26 (5)	28 (6)	25 (4)	23 (2)	32 (10)
	DM750	33 (6)	34 (6)	36 (10)	32 (7)	32 (5)	29 (5)	31 (7)	33 (10)	38 (8)
	M250	42 (7)	57 (11)	59 (13)	59 (15)	56 (13)	58 (14)	51 (12)	NM	NM

With the 500 µg/kg dose, no statistically significant cardiovascular changes were seen when compared to the baseline at five minutes. Mean arterial pressures were significantly reduced from 10 to 90 minutes and heart rates were below baseline values from 30 minutes. Three MAP measurements were below 60 mmHg, with the lowest value being 58 mmHg. These were all from the same dog between 20 and 45 minutes. On the other hand, the cardiac index and oxygen delivery were maintained at baseline levels for the duration of the observational period. No ECG abnormalities were seen.

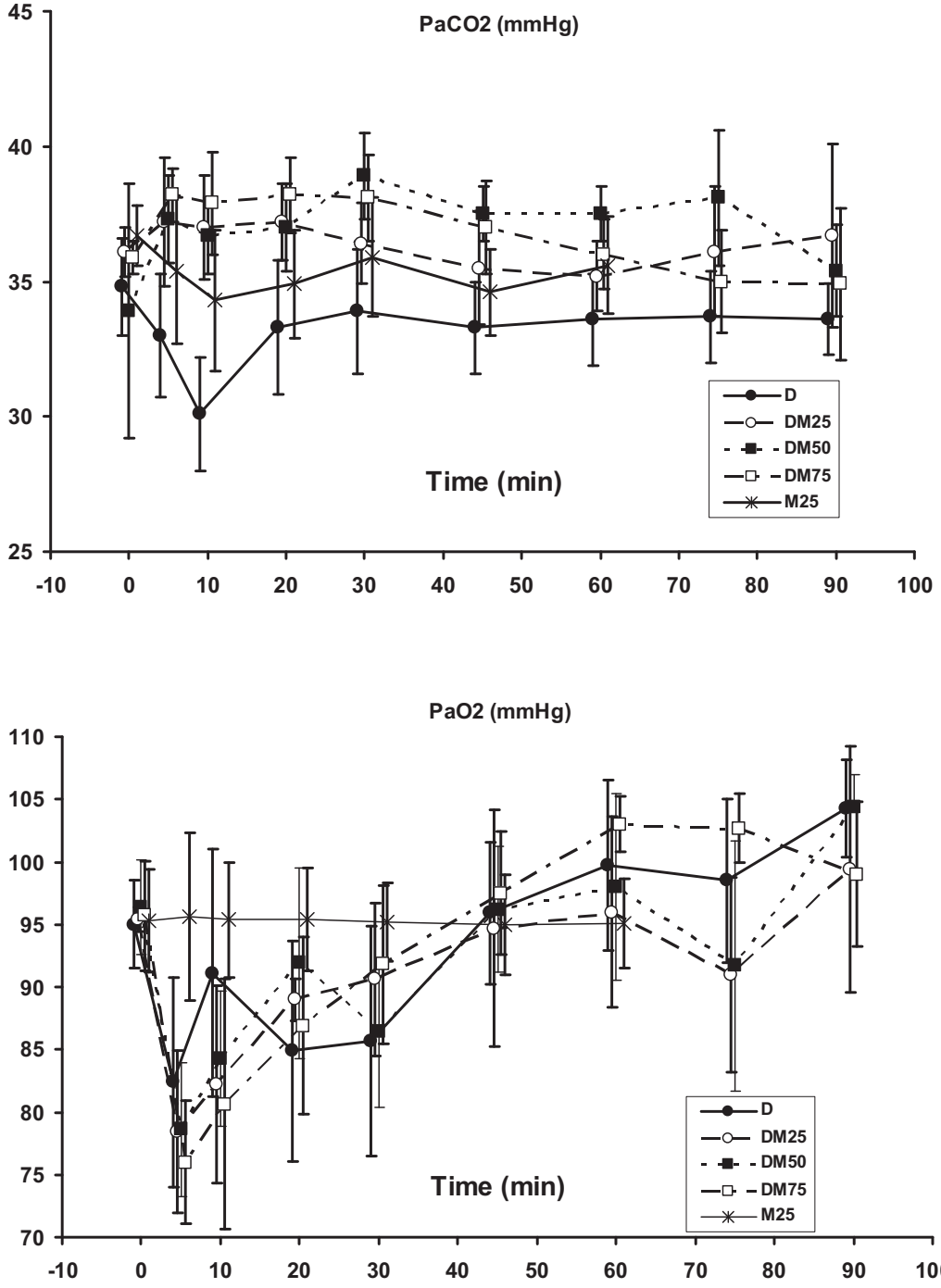
With the highest dose of 750 µg/kg, no significant differences in heart rates from the baseline were seen during the observational period. However, mean arterial pressures were reduced from 5 until 75 minutes, with one dog having values between 55–59 mmHg between 10 to 45 minutes (this was the same dog that had the low values with the previous doses). No significant difference in either cardiac index or oxygen delivery was observed compared to the baseline, while systemic vascular resistance was reduced between 20 and 60 minutes. No ECG alterations were observed.

5.2 Respiratory effects

5.2.1 Respiratory effects of dexmedetomidine (II)

Dexmedetomidine reduced respiration rates in study II (Table 3) when given alone along with a moderate but statistically significant early decrease in PaO_2 with the dogs breathing room air (Figure 5). No significant differences in $PaCO_2$ were observed when compared to the baseline (range 27.7–41.1 mmHg). Consequently, an elevation in alveolar-to-arterial oxygen partial pressure differences was observed. However, arterial oxygen saturations remained above 90% at all times, except for two dogs that had a PaO_2 value below 60 mmHg between 10 and 30 minutes. PaO_2 and $P(A\text{-}a)O_2$ returned to baseline levels from 45 minutes. Inconsequential, but statistically significant decreases in arterial pH and HCO_3^- concentrations were also observed. Arterial blood lactate levels rose compared to the baseline, although no single value reached >2 mmol/l.

Figure 5. Arterial partial pressures of carbon dioxide ($PaCO_2$) and oxygen (PaO_2). Error bars indicate 95% confidence intervals



5.2.2 Respiratory effects of MK-467 (II)

MK-467 had no significant effect on the respiration rate when administered alone (Table 3). Furthermore, no changes in PaO_2 or $PaCO_2$ were seen (Figure 5). Arterial pH and lactate concentrations showed a decreasing trend, reaching statistical significance at some time points, as did the HCO_3^- concentrations.

5.2.3 Respiratory effects of dexmedetomidine combined with MK-467 (II)

Respiratory rates tended to be somewhat higher than with dexmedetomidine alone (Table 3). MK-467 did not improve PaO_2 values at any dose level, but early arterial oxygen tensions were instead slightly lower when compared to dexmedetomidine alone (Figure 5). The differences did not reach statistical significance at any time point. $PaCO_2$ was significantly higher with the two higher dose levels of MK-467 at some time points when compared to dexmedetomidine. However, no $PaCO_2$ values above 45 mmHg were detected with any treatment including the antagonist. Changes in $P(A\text{-}a)O_2$ were similar between all treatments that included dexmedetomidine. Small albeit statistically significant reductions in arterial pH were seen with every MK-467 dose, although the lactate levels decreased with 500 and 750 µg/kg when compared to the baseline and were significantly lower than with dexmedetomidine alone (Table 3).

Table 3. Respiratory rates (RR), arterial blood gases and acid-base parameters from study II (mean (CI95)). NM = not measured

Parameter	Treatment	Baseline	5 min	10 min	20 min	30 min	45 min	60 min	75 min	90 min
RR (1/min)	D	18.8 (5.3)	8.8 (3.6)	7 (1)	6 (1.3)	8 (1.3)	8.5 (1.2)	9.8 (2.2)	9.8 (2.7)	12 (2.2)
	DM250	18 (2.2)	11.5 (3.2)	11 (4.6)	10.8 (4.1)	9.3 (3.4)	10 (2.9)	10 (2.8)	9.3 (1.4)	9.2 (0.8)
	DM500	17 (3.6)	11 (3.4)	10.5 (4)	8.8 (2.4)	9.3 (3)	10 (2.5)	10.3 (2.2)	10.6 (3)	9.3 (0.7)
	DM750	18.3 (6.4)	13.3 (3.4)	10 (2.5)	9.3 (3)	9.3 (2.7)	10.5 (1.8)	11 (2.3)	12.8 (3.6)	12 (5.7)
	M250	18.3 (4.5)	20 (3.8)	20.3 (3.6)	20.7 (5.1)	21.6 (7.3)	21.7 (3.9)	19 (5.3)	NM	NM
pH	D	7.38 (0.01)	7.37 (0.02)	7.38 (0.02)	7.36 (0.02)	7.36 (0.02)	7.36 (0.01)	7.36 (0.01)	7.36 (0.01)	7.37 (0.01)
	DM250	7.38 (0.01)	7.36 (0.01)	7.36 (0.01)	7.36 (0.01)	7.36 (0.01)	7.36 (0.01)	7.36 (0.01)	7.36 (0.02)	7.35 (0.02)
	DM500	7.38 (0.01)	7.34 (0.01)	7.35 (0.01)	7.35 (0.01)	7.34 (0.01)	7.35 (0.01)	7.35 (0.01)	7.35 (0.02)	7.36 (0.01)
	DM750	7.38 (0.01)	7.33 (0.01)	7.33 (0.01)	7.33 (0.01)	7.34 (0.01)	7.35 (0.02)	7.36 (0.02)	7.37 (0.02)	7.36 (0.02)
	M250	7.38 (0.01)	7.36 (0.02)	7.36 (0.01)	7.36 (0.01)	7.35 (0.02)	7.36 (0.01)	7.35 (0.01)	NM	NM
$P(A\text{-}a)O_2$	D	14.7 (2.9)	29.3 (7.7)	23.9 (8.9)	26.4 (7.2)	25 (7.9)	15.4 (5)	11.2 (6.4)	12.3 (5.5)	6.7 (3.2)
	DM250	12.9 (3)	27.9 (6.5)	24.6 (6.8)	18.7 (3.2)	16.7 (5.2)	13 (6.8)	12.2 (6.2)	14.1 (7.1)	10.2 (9.9)
	DM500	14.3 (4.6)	28.2 (5.5)	23.2 (4.4)	17.4 (4.6)	18.5 (4.9)	10.3 (4.6)	10.4 (6.9)	13.9 (7.9)	3.9 (1.5)
	DM750	12.5 (3.8)	29.7 (4.1)	25.5 (8.1)	18.8 (5.2)	14.1 (4.7)	9.6 (4)	4.9 (2.6)	6.4 (2.3)	11.8 (7.8)
	M250	16.3 (4.7)	14.3 (4.4)	17.7 (3.6)	17.1 (4)	17.5 (2.8)	20.2 (3.5)	18.8 (2.8)	NM	NM
HCO_3^- (mmol/L)	D	20.1 (0.9)	18.7 (0.8)	17.3 (0.9)	18.3 (0.8)	18.7 (0.4)	18.4 (0.7)	18.6 (0.7)	18.7 (0.8)	18.9 (0.6)
	DM250	20.7 (0.5)	20.5 (0.8)	20.4 (0.8)	20.7 (0.6)	20.3 (0.7)	20.1 (0.9)	20.1 (0.7)	20.3 (1)	20.2 (1.2)
	DM500	20.6 (0.6)	19.8 (0.9)	19.7 (0.7)	19.9 (0.6)	20.3 (0.5)	20.1 (0.3)	20.1 (0.3)	19.5 (1.3)	19.4 (0.4)
	DM750	20.6 (0.7)	19.8 (0.8)	19.6 (1)	19.8 (0.6)	19.8 (0.6)	19.8 (0.4)	19.6 (0.3)	19.6 (0.3)	19.2 (0.5)
	M250	21 (0.3)	19.4 (0.9)	18.9 (1.1)	19.2 (0.7)	19.4 (0.6)	18.9 (0.6)	19.3 (0.6)	NM	NM
ABE (mmol/L)	D	-3.9 (0.7)	-5.1 (0.8)	-6.2 (0.8)	-5.7 (0.6)	-5.4 (0.4)	-5.7 (0.6)	-5.4 (0.5)	-5.4 (0.7)	-5.1 (0.6)
	DM250	-3.2 (0.6)	-3.9 (0.6)	-3 (2.2)	-3.7 (0.6)	-4.2 (0.6)	-4.3 (0.6)	-4.2 (0.6)	-4.1 (1)	-4.4 (1.1)
	DM500	-4.7 (2.7)	-4.9 (0.8)	-4.9 (0.7)	-4.7 (0.5)	-4.5 (0.5)	-4.6 (0.5)	-4.6 (0.4)	-5.4 (1.8)	-4.9 (0.3)
	DM750	-3.5 (0.8)	-5.1 (0.9)	-5.3 (1)	-5.1 (0.7)	-5.1 (0.7)	-4.7 (0.5)	-4.9 (0.5)	-4.6 (0.5)	-5 (0.3)
	M250	-3.2 (0.3)	-4.8 (0.6)	-5.3 (0.9)	-5.1 (0.5)	-5 (0.4)	-5.4 (0.5)	-5.1 (0.5)	NM	NM
Lactate (mmol/L)	D	0.9 (0.2)	1.1 (0.3)	1.2 (0.3)	1.2 (0.2)	1.3 (0.2)	1.3 (0.2)	1.4 (0.1)	1.4 (0.1)	1.3 (0.2)
	DM250	0.9 (0.2)	0.8 (0.2)	0.8 (0.3)	0.8 (0.3)	0.8 (0.3)	0.8 (0.3)	0.3 (0.3)	1 (0.3)	0.9 (0.3)
	DM500	0.7 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.6 (0.1)	0.5 (0.1)	0.5 (0.1)
	DM750	0.8 (0.2)	0.6 (0.2)	0.6 (0.2)	0.6 (0.2)	0.6 (0.2)	0.6 (0.2)	0.7 (0.2)	0.7 (0.2)	0.8 (0.2)
	M250	0.9 (0.1)	0.8 (0.4)	0.7 (0.2)	0.6 (0.2)	0.6 (0.1)	0.7 (0.3)	0.4 (0.1)	NM	NM

5.3 Plasma drug concentrations

5.3.1 Plasma concentrations of dexmedetomidine (IV)

In study IV, MK-467 had a marked influence on the plasma concentrations of 10 µg/kg of IV dexmedetomidine (Table 4). Regardless of the dose of MK-467, plasma concentrations were significantly reduced when compared to dexmedetomidine alone. Statistical significance was reached from 10 to 60 minutes with 250 µg/kg of MK-467, from 5 to 60 minutes with 500 µg/kg of MK-467 and from 5 to 90 minutes with 750 µg/kg of MK-467. Consequently, the $AUC_{0–90}$ with dexmedetomidine alone was significantly larger when compared to treatments including the antagonist. Similarly, the calculated volumes of distribution and clearance were higher with MK-467. Neither the distribution nor terminal half-life estimates differed significantly between treatments. On visual inspection of the data, however, the distribution of dexmedetomidine from the central compartment gave an impression of being more rapid when the drug was co-administered with MK-467. Modeling after dexmedetomidine alone provided rather poor fits with the employed simple two-compartment model. This resulted in poor accuracy and wide scatter of the distribution half-life estimates.

5.3.2 Plasma concentrations of MK-467 (IV)

Plasma concentrations were successfully determined in five dogs. The volume of distribution was estimated at 0.41 (0.13) L/kg. Simultaneously administered dexmedetomidine had no significant effect on the AUC_{0-60} of MK-467, while a non-linear dose exposure was evident between the two higher doses of MK-467. Plasma concentrations are summarized in Table 4.

Table 4. Concentrations of dexmedetomidine and MK-467 in plasma (mean (CI95%)).

Time (min)	1	3	5	10	20	30	45	60	90
Treatment	**Concentrations of dexmedetomidine in plasma in eight dogs (ng/mL)**								
D	15.3	11.6	11.5	9.4	6.4	4.7	3.5	2.7	1.5
	(4.2)	(2)	(2.1)	(1.9)	(1.2)	(0.8)	(0.6)	(0.4)	(0.3)
DM25	12.6	9.4	7.3	4.4	2.8	2	1.5	1.1	0.8
	(1.8)	(0.7)	(0.7)	(0.7)	(0.4)	(0.4)	(0.2)	(0.2)	(0.2)
DM50	12	8.2	6	3.6	2.3	1.8	1.2	1	0.7
	(1.8)	(1)	(0.7)	(0.5)	(0.4)	(0.3)	(0.2)	(0.2)	(0.2)
DM75	10.5	8.3	5.8	3.4	2.4	1.8	1.5	1.1	0.7
	(2.1)	(2.8)	(1)	(0.6)	(0.4)	(0.3)	(0.3)	(0.2)	(0.2)
	Concentrations of MK-467 in plasma in five dogs (µg/mL)								
M25	1.44	0.93	0.7	0.56	0.45	0.36	0.29	0.22	NM
	(0.26)	(0.27)	(0.3)	(0.23)	(0.19)	(0.16)	(0.12)	(0.11)	
DM25	1.81	1.0	0.69	0.55	0.37	0.31	0.28	0.23	0.16
	(0.47)	(0.31)	(0.24)	(0.14)	(0.11)	(0.1)	(0.08)	(0.1)	(0.06)
DM50	2.89	1.67	1.46	1.25	0.88	0.7	0.63	0.45	0.41
	(0.72)	(0.66)	(0.59)	(0.41)	(0.36)	(0.4)	(0.34)	(0.25)	(0.26)
DM75	2.74	1.62	1.55	1.25	0.91	0.87	0.65	0.57	0.4
	(0.55)	(0.39)	(0.75)	(0.37)	(0.34)	(0.45)	(0.31)	(0.27)	(0.27)

NM = not measured

5.4 Clinical sedation (I, III)

In study I, MK-467 did not significantly influence the composite sedation scores during individual time points; nor did it affect the area under the time–sedation curve. Time to lateral recumbence after drug administration was similar with both treatments (mean (CI95)): 81 (41) seconds with D 5 and 88 (44) seconds with D 5 + MK 250). The degree of sedation decreased rapidly after atipamezole with both treatments. Four dogs scored a CSS > 0 (range 1–5) with D 5, and all dogs had a CSS score of zero with D 5 + MK 250 at the end of the observational period.

In study III, MK-467 did not have a sedative or a stimulating effect when administered alone. After all treatments that included dexmedetomidine, each dog became moderately or deeply sedated. The sedation scores were lower when dexmedetomidine was combined with MK-467, reaching statistical significance with 250 and 500 µg/kg. However, bioequivalence between dexmedetomidine and combinations with all doses of MK-467 was reached for areas under time–sedation curve calculated for the composite sedation scores. Confidence intervals for the areas under the time-sedation curves are presented in Table 5.

Table 5. Confidence intervals of the calculated areas under time-sedation (studies I and III) and time-concentration (study IV) curves for sedation and plasma concentrations of dexmedetomidine. Dexmedetomidine/MK-467 combinations are compared to dexmedetomidine alone.

Study	DOP (min)	Treatment	AUC_{CSS}	AUC_{RATIO}	AUC_{RATIO} (CI95)	AUC_{RATIO} (CI97.5)
I (n=6)	40	D5	13.6			
		DM25	12.7	0.93	0.78 – 1.08	0.76 – 1.11
			AUC_{REC}			
	20	D5	7.0			
		DM25	5.5	0.79	0.42-1.16	0.37-1.21
III (n=8)	90		AUC_{CSS}			
		D10	11.1			
		DM25	9.3	0.84	0.76 – 0.92	0.75 – 0.93
		DM50	9.7	0.87	0.75 – 1.0	0.73 – 1.01
		DM75	8.8	0.79	0.71 – 0.87	0.71 – 0.88
IV (n=8)	90		AUC_{DEX}			
		D10	422			
		DM25	215	0.51	0.42 – 0.6	0.41 – 0.61
		DM50	188	0.46	0.38 – 0.54	0.37 – 0.56
		DM75	189	0.46	0.38 – 0.54	0.37 – 0.56

DOP = duration of observation/sampling period
AUC_{CSS} = area under time-sedation curve / DOP
AUC_{REC} = area under time-sedation curve after 50 µg/kg of atipamezole (IM) / DOP
AUC_{DEX} = area under time-concentration curve of dexmedetomidine
AUC_{RATIO} = $AUC_{COMBINATION}$ / $AUC_{DEXMEDETOMIDINE}$
CI95 and CI97.5 = 95% and 97.5% confidence intervals for AUC_{RATIO}

6. Discussion

6.1 Methodological considerations

The dogs in study I were in good general health, although they already aged from 9 to 11 years during the experiment. Thus, both the cardiovascular and sedation outcomes from this study are not necessarily comparable with studies II–IV, in which eight young beagles were used. The elimination rate of dexmedetomidine has been suggested to be higher in juvenile beagles (3-4 months) than in adults (> 2 years) (EMA, 2002). In aging humans, a reduced vasoconstrictive effect in leg musculature has been demonstrated with dexmedetomidine (Smith et al., 2007). No studies on the effects of ageing on the hypnotic potency of dexmedetomidine were located, although Fragen and Fidgerald (1999) reported a lower sevoflurane MAC-sparing effect of dexmedetomidine in older humans when compared to the previously documented isoflurane MAC-sparing percentage (Aantaa et al., 1997). The younger beagles used in studies II–IV obviously had less time to adapt to the experiments, and while every effort was made to minimize the effect of the methods on the outcomes, baseline cardiopulmonary parameters might well have been influenced by the mere activity of the animals. In fact, in study II, baseline mean arterial pressures were higher than expected for resting dogs.

In study I, the pulse rates after treatments were compared with the resting heart rates obtained by Holter-monitoring. As these two parameters are not necessarily analogous, ECG monitoring during the treatment period would have allowed more appropriate comparisons to be made. However, by definition, the pulse rate cannot be higher than the heart rate. Thus, the heart rate would at worst have been underestimated during sedation after either treatment.

In studies I and III, bioequivalence was demonstrated according to the veterinary guidelines provided by the European Medicines Agency (EMA 2001). As these requirements were primarily designed to form the basis for approval of generic applications and thus mainly focused on the pharmacokinetic behavior of two products that are presumed identical, the use of these guidelines to accept or reject bioequivalence between two or more pharmacodynamic outcomes might not have been an optimal test. Furthermore, the CI90% acceptance interval (0.8–1.25) suggested by the EMA was not necessarily applicable to the hypothesis of the

present studies (bioequivalence in sedation). For example, the World Health Organization recommends customized acceptance intervals that should be justified both statistically and clinically in order to find any relevant difference with reasonable sensitivity (WHO, 2009). The WHO also recommends the use of CI95% instead of CI90% for equivalence studies. However, specific acceptance intervals would have required a consensus on a clinically relevant difference, and as the composite sedation score used in the studies has not been appropriately validated, EMA guidelines provided established limits for assessing the potential similarity between treatments. In any case, clinical bioequivalence between treatments should be accepted with caution.

Power analyses were performed for primary and selected secondary outcomes prior to all studies. Eight dogs were chosen for studies II–IV, as compared to six in study I, in an effort to detect smaller significant differences, especially during the later stages of both the cardiopulmonary and sedation monitoring. As 95% confidence intervals were calculated for most parameters, it became clear that some differences that did not reach statistical significance might still remain clinically important. This was especially observed when arterial partial pressures of oxygen were compared between treatments. The use of harsh *post hoc* corrections, such as the Bonferroni, probably highlighted this effect with parameters that had wide standard deviations. On the other hand, false positive results were less likely, which is commonly accepted as a more appropriate approach.

The main investigator was not blinded to the treatments, but as the cardiopulmonary parameters were numerical and, for the most part, recorded automatically by the use of collecting software, it was deemed as an acceptable flaw and thought not to influence the outcomes. A second investigator assessing subjective central effects was kept unaware of the treatment given and cardiopulmonary monitoring parameters, which fulfilled the criteria of blinding. However, as the administration of MK-467 alone in study III resulted in no apparent sedation, blinding was not possible.

Placebo treatments were not used in any study. Dexmedetomidine acted as a positive control in all studies, and as its cardiovascular and central effects were well known before-hand to be supraphysiological, the lack of a negative control was thought unnecessary, especially as comparisons with the baseline were also carried out. However, we cannot consequently rule out any additive or inhibitory effect

generated either from the study subjects or the settings on any parameter investigated.

In studies II–IV, only six dogs were investigated when MK-467 was administered alone. This decision was made during the experiments, as it became clear that both the central and cardiovascular outcomes were markedly different from all treatments including dexmedetomidine. While the resulting difference in group size induced some difficulties in treatment comparisons, randomization was not affected.

A considerable methodological flaw in study IV was the short duration of sampling for plasma drug concentrations. The authors had previous experience in pharmacokinetic studies with dexmedetomidine in dogs suggesting that more than 120 minutes should be allowed for proper elimination of the parent compound (Kuusela et al., 2000). That said, the differences in plasma concentrations between treatments were large enough to reliably show the effects of MK-467 during the clinically most important first hour after drug administration. Within the same study, plasma concentrations of MK-467 were successfully analyzed in only five dogs, which yielded large deviations. Furthermore, no efforts were made to study the possible chemical interaction between dexmedetomidine and MK-467 when mixed together in a single syringe prior to administration. Thus, while no macroscopic changes such as precipitates or color changes were observed, both the stability and integrity of the mixture should be confirmed by vigorous quality assessments to rule out any potential chemical incompatibility. However, the pharmacodynamic dose-response of the treatments in the present study was logical and repeatable.

As the results for studies II–IV were yielded from the same series, different methods might have affected one another. For example, sedation assessments provided a stimulus that may well have influenced the hemodynamic parameters. Similarly, the mechanical noise from the infusion pump collecting waste arterial blood via the lithium sensor during cardiac output measurements could have affected the degree of sedation. Moreover, noise from the surroundings was impossible to standardize during the experiments. In effort to minimize these deviations, sedation scoring was carried out immediately prior to the cardiac output measurements. Furthermore, the conditions did not differ between treatments, and background noise was minimized with appropriate announcements to staff working adjacent to the research room.

Instrumentation for studies II–IV was carried out under sevoflurane anesthesia. After mask induction of anesthesia, the percentage of sevoflurane (in oxygen) varied between 2–3.5% during arterial and central venous catheter placement, which usually took less than one hour from induction to extubation. Arterial blood pressures were indirectly measured by Doppler and a capnograph was used. No relevant hypo- or hypertension or hypo-/hypercapnia was detected during the instrumentation period. Although the instrumentations were uneventful, it is not possible to exclude any effect on the results of the studies, even if a minimum of 60 minutes was allowed for recovery between extubation and baseline recordings. Recoveries after sevoflurane anesthesia have been reported to be smooth and rapid in dogs (Johnson et al., 1998), and although major signaling pathways in the canine CNS are affected, the duration of effect appears short and linearly dose-dependent (Stucke et al., 2005a and b). Subjectively, all dogs were adequately recovered and showed no apparent deviations in their behavior prior to baseline measurements.

6.2. Cardiovascular effects

MK-467 dose-dependently attenuated or prevented all the relevant hemodynamic alterations induced by dexmedetomidine in this study. This effect was mediated via peripheral antagonism of α_2-adrenoceptors and proved to be consistent and repeatable throughout the study with simultaneous intravenous administration. The cardiovascular indices, such as arterial blood pressure, systemic vascular resistance and heart rate, clearly indicated that the reduction in early afterload by the antagonist was the main single factor that allowed for preserved cardiac function when compared to dexmedetomidine alone. Most importantly, the cardiac index and oxygen delivery were maintained throughout the observational period in study II with dexmedetomidine/MK-467 combinations, and the early cardiovascular disturbances, including the incidence of arrhythmias, were either significantly reduced or completely abolished by increasing doses of the antagonist. These findings are in accordance with other studies on dogs performed with the antagonist and either dexmedetomidine or medetomidine, even though the drug administration protocols were markedly different (Pagel et al., 1998; Enouri et al., 2008).

It is notable that comparisons with studies using racemic medetomidine need to be approached with some caution, as levomedetomidine has been demonstrated to

have potential agonistic activity at the α_1-adrenoceptors and some antagonism at the α_2-adrenoceptors (Schwinn et al., 1991; Kuusela et al., 2000 and 2001a). Furthermore, as the α_2:α_1 specificity of MK-467 is reported to be significantly lower than for either agonist drug, some antagonism at the peripheral α_1-adrenoceptors might have been attributed to its vasodilatatory action (Clineschmidt et al., 1988; Virtanen et al., 1988; Vargas and Gorman, 1995). However, acepromazine, an α_1-adrenoceptor antagonist tranquilizer, had only a small attenuating effect on dexmedetomidine-induced hypertension in dogs (Alvaides et al., 2008). Nevertheless, the marked improvements in the cardiac index and oxygen delivery by MK-467 seem to be applicable for both medetomidine (Enouri et al., 2008) and the dextroisomer in dogs.

After the initial dose-dependent attenuation of the dexmedetomidine-induced alterations in hemodynamic performance by MK-467, a trend towards reductions in both HR and MAP was observed with each combination treatment. This phenomenon would easily be explained by central agonism of α_{2A}-adrenoceptors by dexmedetomidine, leading to central sympatholysis and decreases in vasomotor tone, as previously shown in knock-out mice (Macmillan et al., 1996; Link et al., 1996) and in dogs (Pagel et al., 1998). While no severe hypotension was observed in the present study, the centrally mediated hypotension might readily be highlighted with more multimodal techniques involving cardiopulmonary depression by, for instance, induction drugs or inhalant anesthetics (Kuusela et al., 2001b and 2003; Bennett et al., 2011). Consequently, although the 50:1 dose ratio in study II provided the most stable hemodynamic performance, it might not be optimal when further combined with other sedatives, analgesics or general anesthetics. Thus, multimodal approaches need to be evaluated separately in order to determine a safe and efficacious dose range and ratio.

In study I, in which a lower dose of dexmedetomidine was used, MK-467 at a 50:1 dose ratio was not able to completely reverse the reduction in pulse rates, although the pulse rates during dexmedetomidine/MK-467 sedation were bioequivalent with the resting heart rates obtained via Holter monitoring. This could reflect differences in the study population and experimental setting when compared to study II, in which the same dose ratio, although higher absolute doses were administered. The dogs used in study I were older and more accustomed to handling, and as the methods were kept to an absolute minimum and the experiment was conducted within the

kennel they were housed in, physiological stress responses and agitation probably had little to no effect on the dose responses. As with all sedatives, a quiet and calm environment is necessary to achieve maximal dose effects (Kramer et al., 1996). Thus, general sympatholysis after dexmedetomidine might have been more accentuated than in study II, allowing for a more profound centrally mediated bradycardia. Unfortunately, as factors such as blood pressure, cardiac output and catecholamine concentrations were not measured during study I, alternate explanations such as different absolute dose effects when lower doses of dexmedetomidine are used or the effects of aging cannot be ruled out. It would be very unlikely that a lower dose of dexmedetomidine would have induced more severe cardiovascular effects (Pypendop et al., 1998; Kuusela et al., 2000), unless the absolute dose of MK-467 was insufficient to significantly antagonize the peripheral α_2-adrenoceptors. Overall, MK-467 acted predictably in its effects on the cardiovascular outcome during the studies, which facilitates the design of future experiments and the potential transfer to use in clinical patients.

When administered alone, MK-467 induced a marked increase in heart rates coupled with a moderately decreased systemic vascular resistance. As α-adrenoceptors should have a minimal direct chronotropic effect on the myocardium, the tachycardia was probably mediated via a physiological, reactive compensatory mechanism in response to the systemic vasodilation by the antagonist drug, as previously demonstrated in rats (Szemeredi et al., 1989). While detailed interspecies comparisons of the cardiovascular dose response have not been conducted to our knowledge, the increases in heart rates after intravenous administration seem to be less in rats and humans than in dogs (Szemeredi et al., 1989; Schafers et al., 1992; Sciberras et al., 1994; Pagel et al., 1998). This finding is in accordance with previous suggestions that canines would be more sensitive than humans to the action of drugs acting on vascular α_2-adrenoceptors (Flacke et al., 1990; Bloor et al., 1992a and b). In any case, the cardiovascular outcome after intravenous MK-467 administered alone might have detrimental effects in hypovolemic and/or vasodilated dogs (e.g. sepsis) due to the further increase in the vascular volume. As the dogs in this study were healthy and assumed normovolemic, unchanged central venous pressure would not necessarily have adequately reflected the systemic redistribution of blood or the remaining reserve capacitance of, for instance, either venous or splanchnic vasculature (Gelman 2008). On the other hand, the decrease in afterload

might be beneficial for some patient categories, such as in advanced chronic cardiac valvular disease, where blood regurgitation and congestion might be less with lower systemic vascular resistance (Atkins et al., 2009). However, further studies would be necessary to investigate the effects of MK-467 in dogs with various clinical cardiovascular conditions.

Moreover, in clinical anesthesia, the use of vasopressors is a fundamental necessity to correct severe hypotension (Chen et al., 2007; Rosati et al., 2007). As many of the vasopressors used produce their action by inducing vasoconstriction via the peripheral α_1-adrenoceptors located in vascular smooth muscle, it would to be prudent to confirm that MK-467 does not significantly attenuate the efficacy of these drugs. Due to its relatively low α_2:α_1 specificity, MK-467 might have some antagonistic activity at the α_1-adrenoceptors, although in rats it was unable to inhibit aortic contraction induced by clonidine, an α_2-adrenoceptor agonist with agonist activity at the α_1-adrenoceptor (Iwanaga et al., 1998; Monteiro et al., 2007). Other theoretical complications such as the 'epinephrine reversal syndrome' (Kaul and Grewal, 1970; Hunyady and Johnson, 2006) seem very unlikely, as the use of adrenaline did not induce hypotension in dogs pre-treated with both MK-467 and dexmedetomidine (Hayashi et al., 1991).

The dose-dependent effect of MK-467 on dexmedetomidine-induced changes in heart rates, mean arterial pressure and cardiac index differed markedly from previous experiments with antimuscarinic drugs. In contrast to the tachycardia and severe hypertension observed in several studies that have included atropine and/or glycopyrrolate before or after either medetomidine or dexmedetomidine (Alibhai et al., 1996; Alvaides et al., 2008; Ko et al., 2001), heart rates and mean arterial pressures remained within acceptable limits with dexmedetomidine/MK-467. Furthermore, cardiac dysrhythmias were abolished with the higher dose ratios in the present study, and the cardiac index was better preserved by MK-467 than previously reported for both atropine and glycopyrrolate (Short 1991; Bloor et al., 1992a; Congdon et al., 2011). Artificially preventing the cardiovascular system from adapting to any given afterload, e.g. by the administration of muscarinic antagonists, leads to non-physiological states where the Frank–Starling mechanism of ventricular–vascular coupling is overruled (Jacob and Kissling, 1989). Forced tachycardia against an increased afterload leads to an increased myocardial workload accompanied by a higher oxygen demand and consumption (Lemke et al.,

1993; Alibhai et al., 1996; Sinclair et al., 2003). Acute or prolonged hypertension can also lead to circulatory disturbances followed by, for example, ocular lesions or cerebrovascular events (Wessmann et al., 2009; Leblanc et al., 2011). Enouri et al. (2008) found little improvement from pre-treatment with glycopyrrolate, even when combined with MK-467, when compared to antagonist alone in dogs receiving medetomidine. While life-threatening bradycardia must obviously be addressed by any mean available, no indication for the routine addition of antimuscarinic drugs to α_2-adrenoceptor agonists was identified from the existing literature. It is probable that MK-467 would provide a more benign strategy to improve the cardiovascular performance of dogs sedated with either medetomidine or dexmedetomidine.

6.3 Respiratory effects

Dexmedetomidine decreased respiratory rates and induced a mild reduction in PaO_2, which is in accordance with previous studies (Vainio 1989; Nguyen et al., 1992; Kuusela et al., 2000 and 2001a). MK-467 was unable to prevent the slight decrease in PaO_2 and elevations in $P(A\text{-}a)O_2$ seen with every treatment including dexmedetomidine. If anything, PaO_2 tended to be slightly more reduced by MK-467 during the first 20 minutes after drug administration, although statistical significance between treatments was not reached. As $PaCO_2$ tended to be higher with the treatments including the antagonist, a moderate increase in hypoventilation could explain the differences in PaO_2 between treatments. On the other hand, respiratory rates were somewhat higher with all dexmedetomidine/MK-467 treatments early on. Thus, the differences in arterial gas tensions might be explained by a reduction in tidal and minute volumes when compared to dexmedetomidine alone. Actually, dexmedetomidine has been reported to enhance ventilation at very high doses (up to 100 µg/kg IV) in conscious dogs, an effect which was already seen with the dose levels used in the present study (Nguyen et al., 1992). In any case, any increase in ventilatory drive was abolished or reversed by concurrent administration of propofol or isoflurane (Nguyen et al. 1992, Kuusela et al., 2001b and 2003).

However, it cannot be excluded that MK-467 either blunted a stimulatory or accentuated an inhibitory effect on respiration induced by dexmedetomidine in conscious dogs that were breathing room air. One possible explanation could be an imidazoline-receptor mediated increase in the venous admixture, as MK-467 does

not possess the necessary chemical structure to act as an antagonist at these receptors (Clineshmidt et al., 1988; Kamibayashi et al., 1995b). Ernsberger et al. (1998b) postulated that activation of imidazoline receptors in the carotid body of cats and rabbits facilitates chemosensory responses to hypoxemia, while α_2-adrenoceptors would inhibit this response. Thus, if such inhibitory effect was prevented by MK-467, the remaining stimulatory effect mediated by dexmedetomidine via imidazoline-linked chemoreceptors might explain the slight but consistent differences between treatments in this study. Sudden increases in SVR have also been associated with increased tidal and minute volumes without affecting respiratory rates in humans suffering from vagal syncope (Taneja et al., 2008). Furthermore, decreases in carotid body perfusion have been implicated as a potential mechanism behind a hyperpneic response (Nimbkar and Lateef, 2005). Consequently, the global hemodynamic changes induced by dexmedetomidine could also account for the differences in $PaCO_2$ levels in the present study.

The mechanism behind reductions in PaO_2 after dexmedetomidine administration remains unclear, and the findings of the present study are suggestive of a pathophysiology mediated by other than α_2-adrenoceptors. Fortunately, as dogs are not very susceptible to α_2-adrenoceptor agonist-mediated hypoxemia when compared, for instance, to small ruminants (Kästner 2006), the moderate decreases in PaO_2 should not be of a major concern in healthy animals. Overall, however, more detailed *in vitro* and *in vivo* efforts are necessary to comprehensively evaluate the effect of dexmedetomidine on the ventilation/perfusion ratio in canines. In any case, dexmedetomidine is unlikely to induce severe hypoxemia in healthy dogs, and MK-467 is as unlikely to improve pulmonary gas exchange in compromised animals. However, despite small differences in PaO_2 between treatments, oxygen delivery was superior with treatments including MK-467 as a consequence of a significantly improved cardiac index when compared to dexmedetomidine alone. Arterial lactate concentrations were also higher without the antagonist, indicating reduced tissue perfusion.

6.4 Plasma drug concentrations

6.4.1 Plasma concentrations of dexmedetomidine

Plasma concentrations of dexmedetomidine administered alone were similar to previous reports, and levels that have been associated with profound sedation in dogs were achieved with all treatments (Kuusela et al., 2000). That said, MK-467 markedly reduced the exposure to dexmedetomidine with each dose ratio, as described by the apparent decreases in $AUC_{0\text{-}90}$. Differences in the cardiac index reported in study II also reflected the significant increase in volumes of distribution and clearance when compared to dexmedetomidine alone. A reduction in blood flow to peripheral vascular beds has been suggested to decrease the distribution of drugs that would otherwise be rapidly spread to tissues (De Paepe et al., 2002). Furthermore, in the present study, a large deviation in the earliest plasma concentrations was observed when dexmedetomidine was administered alone. As an explanation, the immediate distribution within the central compartment might have been incomplete, since the average cardiac output without MK-467 was reduced to less than the estimated circulating total blood volume per minute (Hahn et al., 1942). Curiously, the time when the maximum plasma concentration of detomidine, another α_2-adrenoceptor agonist, was observed in horses ranged from one to six minutes after intravenous administration (Grimsrud et al., 2009). Dexmedetomidine has also been shown to alter its own plasma concentrations in humans as a result of changes in cardiac output. However, the differences between the measured cardiac outputs and several target plasma concentrations were small, and as infusion regimens were used instead of bolus dosing, were likely more descriptive of changes in the elimination clearance (Dutta et al., 2000).

In the present study, the early effect of MK-467 was pronounced, while differences were less than obvious after the immediate distribution phase. While the short sampling time in study IV did not allow for proper comparisons of the elimination clearance rate between treatments, MK-467 did not seem to noticeably influence the later rate of decline of dexmedetomidine concentrations within the central compartment, as indicated by the similarity of the slopes between treatments. This finding would be consistent with previous suggestions concerning the importance of biotransformation in the elimination pathway (Salonen et al., 1989; Salonen and

Eloranta, 1990; Duhamel et al., 2010). Kaivosaari et al. (2002) showed a lower *N*-glucuronidation rate in dog liver microsomes when compared to humans, which might further implicate hepatocellular phase II metabolic activity as the rate-limiting step in the terminal elimination rate of dexmedetomidine in healthy canines rather than the absolute degree of hepatic perfusion.

On the other hand, it would be inappropriate to assume that the clearance of dexmedetomidine would be unaffected by its negative effects on cardiovascular function. In fact, Salonen et al. (1995) postulated that atipamezole enhanced the clearance of medetomidine in dogs by restoring hepatic perfusion. The alteration between treatments in the disposition of dexmedetomidine in study IV could just as well have reflected differences in cardiac output and the consequent rate of hepatic clearance, which were most likely augmented by MK-467. However, contemporaneous increases in both the distribution (i.e. tissue perfusion) and metabolic clearance (i.e. hepatic perfusion) might best explain the lower plasma concentrations seen with dexmedetomidine/MK-467 throughout the sampling period. Importantly, and especially since only the parent compound concentrations were analyzed, it was not possible to determine the detailed relationship between liver blood flow and the intrinsic hepatic clearance of dexmedetomidine in dogs from the presently obtained results.

A pharmacokinetic model that incorporates the changes in cardiac output induced by dexmedetomidine has recently been introduced (Pypendop et al., 2012). While this model is based on data acquired from cats, parallel modeling would conceivably be valid for dogs as well. On the other hand, the pharmacokinetic behavior of dexmedetomidine seen with MK-467 in the present study would reduce the need for complex modeling, as the cardiovascular performance would remain unaffected.

In any case, the clinical consequence of the alterations in plasma concentrations in this study require further evaluation and would also necessitate detailed description of the pharmacokinetics of MK-467 in dogs, which was also beyond the scope of this current effort. Potential chemical interactions between the two molecules should also be evaluated. Nevertheless, the significant effect on the disposition of dexmedetomidine induced by MK-467 is unlikely to hinder more detailed pharmacokinetic-pharmacodynamic modeling of the combination in future studies.

6.4.2 Plasma concentrations of MK-467

Novel information regarding the pharmacokinetics of MK-467 in dogs was obtained in study IV. While no efforts were made to assess the concentrations of MK-467 within the CNS, the low volume of distribution confirms the hydrophilic nature of MK-467, which supports its preference to remain within the central compartment after IV administration. Furthermore, dexmedetomidine did not noticeably influence the disposition of MK-467, which facilitates both the design of future efforts and the prediction of dose responses. The similar AUC_{0-90} achieved with the two higher doses of MK-467 might derive from moderate cardiovascular differences between the two treatments (i.e. differences in distribution and/or clearance), although the non-linear dose exposure should be confirmed with a more adequately designed pharmacokinetic study.

6.5 Clinical sedation

MK-467 did not have any apparent sedative or stimulatory effect, which would support its lack of effect within the canine CNS. Furthermore, the degree of clinical sedation induced by combinations of dexmedetomidine and MK-467 was bioequivalent with dexmedetomidine alone, at least when assessed according to the EMA guidelines. All treatments including the agonist led to moderate or deep sedation. However, as a reduction in the sedative effects with dexmedetomidine/MK-467 treatments when compared to dexmedetomidine alone was observed, it cannot be claimed that the antagonist had no effect on the central effects of dexmedetomidine. Furthermore, the sensitivity of the composite sedation score applied in studies I and III in recognizing minor differences in the degree of sedation has not been properly evaluated. Consequently, the clinical significance of the differences found here should be evaluated and confirmed, if possible, by more objective means.

The lower plasma concentrations of dexmedetomidine attributed to MK-467 might have reduced the availability of the agonist drug in the CNS, thus potentially affecting the degree of sedation. However, radiolabeling studies and/or experiments focusing on drug concentration differences between plasma and the CNS should be conducted to confirm any significant effect of MK-467 on the central distribution of

dexmedetomidine (Lin, 2008). Should this prove to be the case, higher absolute doses of dexmedetomidine should be evaluated, as there is no reason why MK-467 would not prevent the cardiovascular effects as long as suitable dose ratios are chosen. Moreover, despite the low volume of distribution detected in study IV, the peripheral selectivity of MK-467 has not been adequately confirmed in dogs, and it is therefore possible that some portion of the antagonist penetrated through the blood–brain barrier and exhibited a mild antagonistic effect at the α_{2A}-adrenoceptors within the canine CNS.

It is also worth considering that some pathological conditions might also impair the integrity of the canine BBB, such as chronic hypothyroidism, steroid responsive meningitis or trauma (Pancotto et al., 2010; Tipold and Schatzberg, 2010). Thus, the effects of MK-467 in the face of such pathologies might be unpredictable when compared to healthy animals. Furthermore, as the function of the permeability glycoprotein within the BBB can be inhibited by pharmacotherapy (e.g. ketoconazole) or affected by hereditary genetic conditions (e.g. ABCB1/MDR-1 mutation in some dog breeds), further efforts are necessary to properly evaluate both the safety and efficacy of MK-467 in order to find potential deviations from the effects seen in this study (Dowling, 2006; Hugnet et al., 2007). It is currently not known whether MK-467 is a substrate for the canine permeability glycoprotein.

The recoveries after atipamezole were rapid and calm with both dexmedetomidine and dexmedetomidine/MK-467 in study I, although some residual sedation was observed 20 minutes after reversal when the dogs did not receive the peripheral antagonist. While this could be indicative of the lower achieved plasma concentrations of dexmedetomidine in the presence of MK-467, it might also imply greater central bioavailability of atipamezole as, coupled with better tissue delivery via the preserved hemodynamics, MK-467 could have competitively inhibited binding of the central reversal agent to the peripheral α_2-adrenoceptors. It is also possible that any portion of MK-467 crossing the BBB might have acted in synergy with atipamezole. Nevertheless, residual sedation impairs the return to normal physiological function and thus, irrespective of the detailed mechanism of this observation, MK-467 might hasten complete recoveries after dexmedetomidine sedation in dogs. Overall, the degree of clinical sedation induced by dexmedetomidine was inhibited by MK-467 to such a small extent that the benefits from a markedly improved cardiovascular performance cannot be overruled.

6.6 Clinical implications and future prospects

Concerns regarding the cardiovascular effects of α_2-adrenoceptor agonists have previously prevented their use in compromised animals (Murrell and Hellebrekers, 2005). Small doses of both medetomidine and dexmedetomidine have been shown to induce marked reductions in the cardiac index and the consequent oxygen delivery, proving these concerns valid. Prior experiments incorporating antimuscarinic drugs to counteract the characteristic bradycardia have repeatedly led to significant hypertension and increases in the myocardial workload, making this approach inappropriate. In contrast, MK-467, by dose-dependently reversing the peripheral α_2-adrenoceptor-mediated vasoconstriction induced by either medetomidine or dexmedetomidine, could ensure a more physiological cardiovascular function while allowing for the sedative action of either agonist drug. In essence, based on the present results, simultaneous administration of MK-467 would potentially increase the clinical safety margin of α_2-adrenoceptor agonists.

Peripheral α_2-adrenoceptors may have a role in mediating some of the antinociceptive properties of dexmedetomidine (Poree et al., 1998; Tomic et al., 2007; Al-Metwalli et al., 2008). Consequently, MK-467 might impede the quality of analgesia achieved with α_2-adrenoceptor agonists. Therefore, experimental and clinical efforts would be necessary to evaluate any potentially negative influence of MK-467 on the efficacy of both medetomidine and dexmedetomidine in alleviating pain, especially in an acute perioperative setting due to the nature of use of α_2-adrenoceptor agonists in clinical veterinary medicine. A possible attenuating effect of MK-467 on the synergy between, for instance, opioids and α_2-adrenoceptor agonists should also be evaluated in clinical patients (Gursoy et al., 2011).

Many recent efforts in humans have been made to describe the significance of genetic variation in the haplotype expression of different α_2-adrenoceptor subtypes, and the potential consequence for the sensitivity to α_2-adrenoceptor agonists (Muszkat et al., 2005 and 2010; Kurnik et al., 2006 and 2011). While numerous novel variants in genes encoding the different α_2-adrenoceptors of healthy volunteers have been identified, their role in mediating a varying peripheral cardiovascular sensitivity to dexmedetomidine remains to be elucidated. The canine pharmacogenetics of α_2-adrenoceptors remains an undiscovered field of study to date, with many genetically condensed breeds potentially facilitating the discovery of clinically relevant haplotype

variation. Presently, α_2-adrenoceptor subtype-specific drugs are commercially unavailable. The constantly accumulating knowledge of structural differences between receptor subtypes mediating distinct pharmacological effects might guide research and development towards more specific drugs, free of undesirable side or adverse effects.

7. Conclusions

1) MK-467 dose dependently prevented or attenuated the early hemodynamic changes induced by dexmedetomidine in healthy, conscious dogs. Simultaneous intravenous administration of the two drugs proved efficacious and reliable, yielding steady levels of oxygen delivery throughout the studies.

2) MK-467 reduced the plasma concentrations of dexmedetomidine, which might explain the small differences seen in the degree of clinical sedation.

3) Besides a transient sinus tachycardia (accompanied by a reduction in systemic vascular resistance), no clinically observable adverse effect was seen when MK-467 was administered alone.

4) MK-467 decreased the intensity of clinical sedation by dexmedetomidine. Atipamezole successfully reversed the clinical sedation induced by dexmedetomidine/MK-467.

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