Reduction and reversal of the undesirable effects of medetomidine with two $\alpha_2$-adrenoceptor antagonists, vatinoxan and atipamezole, in sedated and ketamine-anaesthetized dogs

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DOCTORAL DISSERTATION
To be presented for public examination, with the permission of the Faculty of Veterinary Medicine, University of Helsinki
in Athena 107 Auditorium, Siltavuoren Penger 3 A, Helsinki
June 1st 2020, at 14 o’clock afternoon
To my four-legged friends
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**ABSTRACT**

$\alpha_2$-Adrenoceptor agonists, such as medetomidine and dexmedetomidine are considered to produce reliable sedation, muscle relaxation, and antinociception when used as sedatives or preanaesthetic medication. One important advantage is that their effects can be reversed with an $\alpha_2$-adrenoceptor antagonist, atipamezole. However, notable cardiovascular depression associated with $\alpha_2$-adrenoceptor agonists limit their safe use to healthy animals. A peripheral $\alpha_2$-adrenoceptor antagonist vatinoxan (also known as MK-467 and L 659’066) has been shown to reduce these cardiovascular disturbances, without markedly affecting sedation after simultaneous use with dexmedetomidine or medetomidine. Vatinoxan is therefore proposed to improve the clinical usefulness of $\alpha_2$-adrenoceptor agonists in dogs.

The main aim of this series of experiments was to investigate the reduction of medetomidine-induced cardiovascular changes with vatinoxan, atipamezole and ketamine, not only in laboratory dogs, but also in healthy client-owned dogs of various breeds in a clinical environment. The secondary aim was to assess the quality of sedation, hypnosis and recovery when these drugs were used together. Two of the studies were performed under laboratory conditions with eight purpose-bred beagle dogs that were instrumented prior to each experiment. Heart rate, mean arterial pressure, central venous pressure, cardiac output and arterial blood gas partial pressures were measured and sedation was assessed. Blood samples were collected for plasma drug concentration analyses. Study I aimed to investigate the ability of atipamezole to reverse the cardiovascular and sedative effects of medetomidine in the presence or absence of vatinoxan. Study II aimed to explore the influence of vatinoxan on the cardiovascular function and quality of anaesthesia and recovery, when the dogs were premedicated with medetomidine and butorphanol, followed by ketamine, and subsequent reversal with atipamezole. In study III the clinical usefulness of vatinoxan was then evaluated in client-owned dogs, sedated with medetomidine and butorphanol combination for non-invasive diagnostic imaging. The need for atipamezole reversal was also monitored. Plasma concentrations of the studied drugs were determined in each study to detect the influence of vatinoxan.

In study I, atipamezole failed to permanently increase heart rate or cardiac index without the presence of vatinoxan when administered intramuscularly 30 minutes after medetomidine. Momentary decrease in mean arterial pressure was observed shortly after atipamezole administration with and without vatinoxan. However, no clinically relevant hypotension was detected. Atipamezole reversed the sedative effect of medetomidine more efficiently with than without vatinoxan, probably due to increased plasma clearance of medetomidine. Relapse into sedation after initial arousal was observed during recovery when vatinoxan was not included in the treatment.

The medetomidine-induced increase in systemic vascular resistance index was attenuated by vatinoxan, with significantly lower values observed throughout the studies I and II in its presence. Medetomidine-evoked bradycardia and decrease in cardiac index were similarly mitigated by
vatinoxan in all of the present studies. In study II, heart rate and cardiac index increased temporarily after ketamine administration and the effect was potentiated by vatinoxan. Ketamine decreased mean arterial pressure and mild hypotension was detected in two out of eight laboratory dogs that were given vatinoxan premedication. Hypotension was abolished by atipamezole administration at 60 minutes following ketamine. Vatinoxan reduced the exposure to ketamine. Therefore, the duration of anaesthesia was shorter in the presence of vatinoxan. The quality of recovery after atipamezole was impaired by nausea and increased need to defaecate with and without vatinoxan.

In study III, intramuscular medetomidine-butorphanol combination with and without vatinoxan provided reliable sedation for diagnostic imaging procedure. Vatinoxan hastened and increased the peak plasma concentrations of intramuscularly co-administered medetomidine and butorphanol. The clinically observed benefit was a faster onset of deeper sedation. Mean heart rate in the treatment group with vatinoxan during the study was approximately 50% higher than in the group without vatinoxan. Atipamezole was required less frequently to reverse medetomidine-evoked sedation when vatinoxan was included in the treatment because of shorter duration of sedation.

To conclude, the two α₂-adrenoceptor antagonists, vatinoxan and atipamezole, interacted favourably in medetomidine-sedated dogs. Together they provided more complete reversal of medetomidine’s effects. While medetomidine-induced cardiovascular changes were transiently attenuated by ketamine and atipamezole, more complete improvement in haemodynamics was observed only in the presence of vatinoxan. Although vatinoxan premedicated dogs were more prone to hypotension during ketamine anaesthesia, heart rate and cardiac index were well-maintained. The clinical usefulness of vatinoxan with medetomidine and butorphanol was demonstrated in a clinical trial. Vatinoxan alleviated the medetomidine-induced bradycardia and the drug combination including vatinoxan provided adequate sedation to complete short, non-invasive procedures. Faster decline in the plasma concentrations of co-administered drugs in the presence of vatinoxan was clinically manifested as shorter duration of sedation and anaesthesia when compared to treatment without vatinoxan.
LIST OF PUBLICATIONS

This thesis is based on the following original publications:


These publications are referred to in the text by their roman numerals (I – III).

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### 1 INTRODUCTION

Procedural and preanaesthetic sedation is often necessary in small animal practice to allow safe, effective and stress-free handling and immobilization of the animal. Balanced anesthesia techniques aim to decrease cardiovascular depression, while maintaining or improving the quality of sedation, degree of antinociception, and providing an anaesthetic-sparing effect.

$\alpha_2$-Adrenoceptor agonists, such as medetomidine or its pharmacologically active enantiomer dexmedetomidine, are commonly combined with different full opioid agonists or the mixed opioid agonist-antagonist, butorphanol, for various clinical procedures and as anaesthetic premedication. However, severe cardiovascular depression attributed to $\alpha_2$-adrenoceptor agonist-induced peripheral vasoconstriction and decrease in sympathetic nervous outflow from the central nervous system, limit the safe use of these combinations, especially in animals with systemic disease. There have been attempts to reduce these unwanted side effects, e.g. by using antimuscarinic agents, but without favorable outcome (Short 1991, Alibhai et al. 1996, Ko et al. 2001, Alvaides et al. 2008, Congdon et al. 2011). During the past decade a peripheral $\alpha_2$-adrenoceptor antagonist, vatinoxan (also known as MK-467), has been actively investigated for simultaneous use with medetomidine in dogs. It has been shown to successfully reduce the adverse cardiovascular effects, without marked effect on initial sedation or analgesia (Honkavaara et al. 2008, 2011, Restitutti et al. 2011, Rolfe et al. 2012, Bennett et al. 2016).

One of the benefits of using medetomidine or dexmedetomidine as sedative agents is their ready reversal with the commercially available $\alpha_2$-adrenoceptor antagonist atipamezole. A review of the literature indicates that there are far fewer studies investigating atipamezole’s cardiovascular and sedation reversal effects when compared to experiments done with medetomidine and dexmedetomidine. Even though, atipamezole is commonly used in clinical practice to shorten the recovery time after procedures and to reverse the adverse cardiovascular effects of $\alpha_2$-adrenoceptor agonists.

In the present studies we hypothesized that:

1. Atipamezole administration would improve cardiovascular performance and hasten the recovery, and that the two $\alpha_2$-adrenoceptor antagonists would interact favorably.
2. Vatinoxan would improve cardiovascular performance by decreasing systemic vascular resistance index and increasing heart rate and cardiac index, without an impact on quality of ketamine anaesthesia and recovery.
3. Intramuscular co-administration of vatinoxan with medetomidine and butorphanol would attenuate bradycardia without impairing the sedative effect of the drug combination, and that atipamezole could be used with reduced dose if needed.
4. In the presence of vatinoxan, the concentrations of intramuscularly co-administered drugs would increase faster during the absorption phase, but also decline faster after the absorption phase has passed.

The following literature survey focuses on clinically relevant pharmacodynamic effects of medetomidine, reduction of its undesirable effects in dogs with dose adjustment and by use of selected drugs. Reversal of medetomidine’s actions with atipamezole in dogs is also reviewed.
2 REVIEW OF THE LITERATURE

2.1 Clinical and pharmacodynamic effects of medetomidine in dogs

Adrenoceptor refers to a membrane-bound receptor that mediates the effects of endogenous epinephrine and norepinephrine in the peripheral and central nervous system. Alpha-adrenergic receptors are divided into $\alpha_1$ and $\alpha_2$ types, distributed widely within central nervous system and peripheral tissues and organs, thus activation of these receptors results in a wide variety of physiological effects. $\alpha_2$-Adrenoceptors are further divided into $\alpha_{2A}$, $\alpha_{2B}$, and $\alpha_{2C}$-subtypes (Bylund 1992).

Medetomidine is a highly selective $\alpha_2$-adrenoceptor agonist, with an $\alpha_2:\alpha_1$ selectivity ratio of 1620:1 (Virtanen et al. 1988) and hence, the clinically observed medetomidine-induced effects are due to agonism and activation of $\alpha_2$-adrenoceptors (Scheinin et al. 1989). Medetomidine contains equal parts of two enantiomers, dexmedetomidine and levomedetomidine, of which dexmedetomidine is the active ingredient that is responsible for the characteristic effects of medetomidine (Savola and Virtanen 1991). Hence, dexmedetomidine is considered to be twice as potent as medetomidine (Virtanen 1989), reflected in a clinical dose of medetomidine that is twice that of dexmedetomidine ($\mu$g m$^{-2}$ or $\mu$g kg$^{-1}$).

2.1.1 Sedation and antinociception

$\alpha_2$-Adrenoceptor agonists are often favoured in veterinary practice because of its ability to produce reliable sedation. The sedative effects are attributed to stimulation of $\alpha_{2A}$-adrenoceptors densely expressed in the noradrenergic nucleus in the pons of brainstem, locus coeruleus, that regulates sleep and wakefulness and modulates sympathetic nervous outflow (Correa-Sales et al. 1992). Since spontaneous firing rates of locus coeruleus neurons correlate with the degree of arousal and vigilance, silencing these noradrenergic neurones by hyperpolarization is a key factor underlying the mechanism of e.g. medetomidine’s sedative action (Scheinin and Schwinn 1992).

When the sedative effects of medetomidine and dexmedetomidine were compared in dogs in two studies, no clinically relevant differences were detected (Kuusela et al. 2000, Granholm et al. 2007). However, in another report medetomidine appeared to produce more intense sedation than dexmedetomidine (Raszplewicz et al. 2013). Failure to achieve desired levels of sedation with $\alpha_2$-adrenoceptor agonists may occur if endogenous catecholamine levels are increased e.g. due to stress, anxiety or pain. Based on clinical experience, this could be explained by an insufficient medetomidine-evoked reduction in excitatory neurotransmitter release within the central nervous system in animals with elevated sympathetic nervous activity (Sinclair 2003).

Intramuscular medetomidine doses of 20-40 $\mu$g kg$^{-1}$ are proposed as optimal for procedural sedation and as anaesthesia premedication in dogs (Clarke and England 1989, Young et al. 1990, Short et al. 1992). Kuusela et al. (2000) observed a similar level of sedation using dexmedetomidine doses of 10 and 20 $\mu$g kg$^{-1}$ in dogs, although the duration of sedation increased with an increasing dose. Pypendop and Verstegen (1998) also concluded that increasing the medetomidine dose only
increased the duration, but not the depth of sedation in dogs. Hence, it was proposed that there is a ceiling effect in the intensity of sedation that cannot be exceeded by further increasing the dose of α2-adrenoceptor agonist (Kuusela et al. 2000).

At sedative doses, the antinociceptive effects of medetomidine are mediated through activation of supraspinal α2-adrenergic mechanisms at the thalamic level, and with high anesthetic doses also at the spinal cord level (Pertovaara et al. 1991). It may be difficult to determine whether the responses to noxious stimuli are attenuated by true antinociception or by decreases in responsiveness due to sedation, as demonstrated in behavioural studies in rats (Pertovaara et al. 1994). In dogs, the antinociceptive and sedative effects of dexmedetomidine were investigated and demonstrated by Van Oostrom et al. (2011) during the administration of a constant rate infusion. It was concluded that sedation was evident at lower plasma dexmedetomidine concentration than required for antinociception (Van Oostrom et al. 2011).

2.1.2 Physiological changes in dogs

2.1.2.1 Haemodynamics and respiration

After medetomidine administration, cardiovascular function is affected by activation of both peripheral and central α2-adrenoceptors. The initial hypertension results from activation of peripheral α2-adrenoceptors in vascular smooth muscles that elicit vasoconstriction. Vasoconstriction causes substantial increase in systemic vascular resistance, that is observed as an elevation of arterial pressures (Autran De Morais and Muir 1995). An increase in arterial blood pressure activates stretch receptors located in the carotid sinus and thoracic aorta (Flacke et al. 1992). They send afferent impulses to the nucleus tractus solitarius in the medulla delivering the message to the dorsal motor nucleus of the vagus, from which parasympathetic nerves are activated to slow the heart rate (Flacke 1992). The central effects of medetomidine in turn decrease sympathetic nervous outflow from the central nervous system, leading to decreased adrenergic activity and bradycardia. Hence, even though the initial peripheral cardiovascular depressant effects decrease over time, the centrally-mediated effects attenuate the increase in heart rate.

Although the precise mechanism for the decrease in cardiac output after administration of α2-adrenoceptors is unknown, the following reasons have been suggested: 1) decreased heart rate and increased afterload, both resulting from increased systemic vascular resistance, 2) decrease in circulatory plasma catecholamine levels leading to reduced inotropy limiting stroke volume, and 3) vasoconstriction of proximal and distal coronary arteries causing myocardial hypoxia and dysfunction as a response (Bloor et al. 1992, Flacke et al. 1993). Direct depressant effects on the canine myocardium by α2-adrenoceptor agonists have not been detected and are considered unlikely (Flacke 1992). Bradyarrhythmias, such as sinus pauses as well as 1st degree and 2nd degree atrioventricular blocks have been reported after administration of both medetomidine and dexmedetomidine (Kuusela et al. 2000).
In addition to its peripheral effects, medetomidine also reduces central sympathetic and augments parasympathetic nervous tone (Xu et al. 1998). As a result, a decline in blood pressure is observed while bradycardia and the decrease in cardiac output nevertheless persist. These changes are indicative of the inhibitory action or resetting of the baroreceptor reflex, and indirect suppression of myocardial activity mediated through reduced catecholamine concentrations (Flacke et al. 1993, Xu et al. 1998). Peripherally mediated arterial hypertension subsides by the centrally mediated reduction in sympathetic nervous activity. The biphasic blood pressure response, an initial increase followed by a decrease, was reported after intravenous administration of medetomidine at doses of 5, 10 and 20 µg kg-1, but was not observed with lower doses (Pypendop and Verstegen 1998). Hence, it was proposed that with low medetomidine doses (1 and 2 µg kg⁻¹) central effects dominate, whereas higher doses produce a stronger stimulation of peripheral α₂-adrenoceptors, overriding the central effects (Pypendop and Verstegen 1998).

All of the aforementioned haemodynamic changes decrease peripheral perfusion and oxygen delivery to the tissues. In anaesthetized dogs, dexmedetomidine causes a redistribution of blood flow to preserve perfusion to vital organs including the heart, brain and kidneys, whereas blood flow to skin, spleen and intestines is decreased (Lawrence et al. 1996, Pypendop and Verstegen 2000).

Administration of medetomidine decreases respiratory rate with minimal effects on arterial blood gas values in dogs (Kramer et al. 1996). Lerche and Muir (2004) reported that besides reducing respiratory rate, medetomidine administered 5 or 10 µg kg⁻¹ intravenously decreased respiratory centre sensitivity and neurorespiratory drive in conscious dogs in response to the increased inspired carbon dioxide. The authors proposed that medetomidine’s respiratory depressant effects are attributed to depression of the central respiratory centres due to activation of α₂-adrenoceptors and decrease in catecholamine activity in the locus coeruleus (Lerche and Muir 2006).

2.1.2.2 Endocrine and gastrointestinal function

Flacke et al. 1993 reported that the dexmedetomidine-induced decrease in central sympathetic outflow is reflected in the dose-dependent fall in plasma catecholamine levels. Administration of 1 µg kg⁻¹ of dexmedetomidine intravenously produced a maximal effect, reducing plasma catecholamines to negligible values, indicative of sympatholysis (Flacke et al. 1993). Medetomidine also reduces plasma insulin levels, thereby increasing plasma glucose concentrations after intramuscular administration of 20, 40 and 80 µg kg⁻¹ (Ambrisko et al. 2005). In mice, the rise in plasma glucose levels was explained by medetomidine’s inhibitory action at pancreatic α₂A-adrenoceptor-mediated insulin release (Fagerholm et al. 2004). Sedation with α₂-adrenoceptor agonists is further associated with increased urine production, and administration of medetomidine intravenously at doses of 10 and 20 µg kg⁻¹ induced diuresis lasting up to 4 hours (Burton et al. 1998). This effect is attributed to central supression of antidiuretic hormone release (Burton et al. 1998, Saleh et al. 2005). Osmotic diuresis resulting from increased glucose concentration in the urine may contribute (Burton et al. 1998). Hence, clinically it is recommended to use medetomidine with caution in diabetic, hypovolemic or dehydrated dogs and when urinary tract obstruction is suspected (Burton et al. 1998, Sinclair 2003).
Vomiting occurs occasionally in dogs after medetomidine administration (Nilsfors et al. 1989, Vainio et al. 1989, Young et al. 1990). In cats emesis is provoked by \(\alpha_2\)-adrenoceptor agonists owing to stimulation of the area postrema that contains the chemoreceptor trigger zone initiating the vomiting (Colby et al. 1981). In general, the gastrointestinal tract is innervated by extrinsic noradrenergic nerves regulating various digestive functions by activation of \(\alpha_2\)-adrenoceptors (Blandizzi 2007). Medetomidine affects the pattern of digestive motility and mucosal secretions (Nakamura et al. 1997). Medetomidine inhibits intestinal motility in the gastric antrum, small intestine and colon and prevents the release of gastrin in the stomach (Maugeri et al. 1994, Nakamura et al. 1997).

2.2 Reduction of the undesirable effects of medetomidine in dogs

Cardiovascular and respiratory complications are major causes of peri-operative anaesthetic deaths in small animals (Brodbelt 2009). Anaesthesia including the \(\alpha_2\)-adrenoceptor agonist xylazine resulted in exceptionally high mortality rate compared to anaesthesia protocols excluding its use (Clarke and Hall 1990). However, when premedication with medetomidine was evaluated in dogs almost two decades later, increased odds of anaesthesia related death were not detected (Brodbelt et al. 2008). It was reported that the the odds of death following medetomidine were similar or lower than odds following premedication with acepromazine (Brodbelt et al. 2008). Increased awareness of the haemodynamic risks involving the use of \(\alpha_2\)-adrenoceptor agonists and better understanding of their use were proposed as an explanation by Brodbelt et al. (2008) for this change. Nevertheless, concerns regarding the adverse cardiovascular effects continue to limit the use of medetomidine in veterinary practice. The pronounced cardiovascular depression restricts the use of medetomidine to healthy dogs or those with mild systemic disease. Hence, there have been many attempts to reduce the undesirable effects of medetomidine in order to improve its clinical usefulness.

2.2.1 Dose adjustment

Medetomidine-induced cardiovascular depression was considered to be dose dependent, hence dose reduction was believed to alleviate the adverse cardiovascular effects. However, this assumption was annulled by Pypendop and Verstegen (1998) demonstrating that haemodynamic changes were evident regardless of medetomidine dose. Cardiac index was reduced by over 50% from baseline even with the lowest medetomidine dose of 1 \(\mu\)g kg\(^{-1}\) (Pypendop and Verstegen 1998). It was observed that maximal cardiovascular changes were present already with 5 \(\mu\)g kg\(^{-1}\) dose and increasing the dose further only minimally exacerbated haemodynamic disturbances, and merely prolonged their duration (Pypendop and Verstegen 1998). Similarly, when medetomidine was administered as constant rate infusions of 1, 2 and 3 \(\mu\)g kg\(^{-1}\) hour\(^{-1}\), reduction of heart rate up to 60% and cardiac output up to 70% were observed (Carter et al. 2010). Since deeper sedation was obtained only with 20 \(\mu\)g kg\(^{-1}\) dose, it was concluded that when medetomidine is used in clinical practice for procedural or preanaesthetic sedation, undesirable cardiovascular changes are always present (Pypendop and Verstegen 1998).
To summarize, reducing the dose of medetomidine shortens the duration of cardiovascular changes without significant effect on the magnitude of haemodynamic depression when clinically relevant medetomidine doses are employed. Since reliable sedation is not achieved at very low doses of medetomidine, this technique cannot be used to avoid its unwanted cardiovascular effects. Rather other sedative, analgesic or anaesthetic drugs should be used in conjunction with medetomidine.

2.2.1.1 Co-administration of butorphanol with medetomidine

Butorphanol is a synthetic, mixed agonist-antagonist opioid exerting most of its analgesic and sedative effects via activity at κ receptors (Raffe and Tranquilli 1989). Butorphanol is often co-administered with medetomidine to enhance both the level and quality of sedation and analgesia. Bartram et al. (1994) observed that the sedative effects of a medetomidine 10 µg kg⁻¹ and butorphanol 0.1 mg kg⁻¹ combination given intramuscularly were similar to those of medetomidine 40 µg kg⁻¹ alone and sufficient for non-invasive diagnostic imaging. The combination of intravenous medetomidine 20 µg kg⁻¹ and butorphanol 0.1 mg kg⁻¹ provided a rapid and smooth sedative effect, longer duration of sedation, as well as greater muscle relaxation and improved analgesia when compared to medetomidine alone (Kuo and Keegan 2004). Similar effects were observed after intramuscular administration of identical drug dosages (Hayashi et al. 1994b). A low dose of medetomidine 1 µg kg⁻¹ combined with butorphanol 0.1 mg kg⁻¹ and administered intravenously produced sedation, whereas neither medetomidine or butorphanol alone produced any detectable sedative effect (Girard et al. 2010). Ko et al. (2000) concluded that the combination of medetomidine (30 µg kg⁻¹) and butorphanol (0.2 mg kg⁻¹) resulted in more reliable and stable sedation than medetomidine alone, but analgesia was not considered sufficient for moderately or severely painful procedures. The authors recommended that medetomidine (10 µg kg⁻¹) combined with butorphanol (0.2 mg kg⁻¹) should be reserved for sedation of dogs undergoing non-painful or only mildly invasive procedures (Ko et al. 1996).

Butorphanol might potentiate the cardiovascular and respiratory depressant effects of medetomidine since it decreases heart rate, cardiac output and arterial partial pressure of oxygen in dogs (Sederberg et al. 1981, Trim 1983). However, differences in cardiovascular depression were not observed by Pypendop et al. (1996) when butorphanol (0.1 mg kg⁻¹) and midazolam (1 mg kg⁻¹) were given intravenously 20 minutes after intramuscular administration of medetomidine (1 mg m⁻² equivalent of 39-46 µg kg⁻¹). This indicated that medetomidine was mainly responsible for the observed cardiovascular changes. The addition of 0.2 mg kg⁻¹ butorphanol did neither adversely affect cardiovascular performance when it was co-administered with 20 µg kg⁻¹ medetomidine given intravenously (Kuo and Keegan 2004). Similarly, when butorphanol 0.1 mg kg⁻¹ was combined with low-dose medetomidine 1 µg kg⁻¹ and given intravenously, the median decrease in heart rate following drug administration was 24% compared to 31% with medetomidine alone (Girard et al. 2010). Hence butorphanol did not appear to add to the medetomidine-induced bradycardic effect (Girard et al. 2010).

To summarize, when medetomidine dose is reduced to shorten the duration of the unwanted cardiovascular effects, co-administration of butorphanol is considered to be beneficial. It potentiates medetomidine’s sedative, muscle relaxing and analgesic effects and also increases the duration of sedation, without adding to the haemodynamic depression.
2.2.2 Pharmacological strategies to attenuate medetomidine-induced cardiovascular adverse effects

Since severe bradycardia induced by $\alpha_2$-adrenoceptor agonists is easy to recognize, there is often a desire to correct bradycardia with drugs that increase heart rate. However, that might not be advantageous if systemic vasoconstriction is simultaneously present. With common medetomidine doses used in clinical practice to sedate dogs intramuscularly or intravenously, initial hypertension is present. In order to prevent or reverse it, drugs that antagonize, or at least alleviate, $\alpha_2$-adrenoceptor mediated vasoconstriction, can be used.

2.2.2.1 Atropine and glycopyrrolate

Two antimuscarinic drugs that are commonly used to treat bradycardia in veterinary medicine are atropine and glycopyrrolate. Their mechanism of action is competitive antagonism of acetylcholine at the muscarinic receptors (Birdsall et al. 1988). Antimuscarinic drugs are effective in preventing the dexmedetomidine and medetomidine-induced decrease in heart rate when given preemptively and increasing heart rate when given concurrently (Short 1991, Alibhai et al. 1996, Ko et al. 2001, Alvaides et al. 2008, Congdon et al. 2011). However, no parallel increase in cardiac output with heart rate has been detected (Congdon et al. 2011). In addition, a significant increase in blood pressure occurs synchronously with increases in heart rate, and deleterious cardiac arrhythmias can be seen (Short 1991, Alibhai et al. 1996, Ko et al. 2001, Alvaides et al. 2008, Congdon et al. 2011).

The hypertensive effects of medetomidine normally trigger a decrease in heart rate, but when antimuscarinic drugs are administered, severe hypertension occurs which, together with an increased heart rate, can cause excessive myocardial oxygen consumption, leading to myocardial hypoxia (Alvaides et al. 2008). *Pulsus alternans* of the arterial waveform, indicative of severe cardiac dysfunction, has been observed in conjunction with high heart rates and blood pressures (Short 1991, Ko et al. 2001). Together these changes were considered to be the result of an imbalance between parasympathetic and sympathetic nervous responses (Short 1991, Ko et al. 2001).

To conclude, although antimuscarinic drugs counteract medetomidine-induced bradycardia, their use does not improve cardiac output, but instead results in prolonged and severe hypertension and potentially detrimental arrhythmias. Hence, the routine use of antimuscarinic drugs with $\alpha_2$-adrenoceptor agonists is considered disadvantageous and is not recommended in dogs (Alibhai et al. 1996, Congdon et al. 2011).

2.2.2.2 Peripheral $\alpha_2$-adrenoceptor antagonist vatinoxan

Vatinoxan attenuates the adverse cardiovascular effects typical of $\alpha_2$-adrenoceptor agonists when administered prior to or simultaneously with the agonist, using either the intravenous or intramuscular administration route (Pagel et al. 1998, Enouri et al. 2008, Honkavaara et al. 2008, 2011, Rolfe et al. 2012, Restitutti et al. 2017). The direct effect of vatinoxan is restricted to peripheral tissues because of its poor lipid solubility (Clineschmidt et al. 1988). Limited penetration across the canine blood-brain barrier is demonstrated by the central nervous system:plasma drug ratio of
approximately 1:50 (Honkavaara et al. 2020). With co-administration of vatinoxan, dexmedetomidine-induced peripherally mediated cardiovascular effects are attenuated without reduction in the level of sedation (Honkavaara et al. 2008, Rolfe et al. 2012). Since vatinoxan does not prevent activation of central α2-adrenoceptors by the agonist, decrease in sympathetic tone and a relative increase in vagal tone leads to decreases in heart rate and cardiac output even in the presence of vatinoxan when compared to an alert, conscious state.

When vatinoxan (100, 200 or 400 µg kg\(^{-1}\)) was administered 30 minutes prior to dexmedetomidine (5 µg kg\(^{-1}\)), and both drugs were given as intravenous infusions over 10 minutes, vatinoxan caused a dose-related decrease in systemic vascular resistance (Pagel et al. 1998). This effect was considered to indicate α2-adrenoceptor blockade within arterial vascular smooth muscles (Pagel et al. 1998). Honkavaara et al. (2011) reported similar findings after intravenous dexmedetomidine (10 µg kg\(^{-1}\)) administered simultaneously with vatinoxan (250, 500 and 750 µg kg\(^{-1}\)). They observed that both systemic vascular resistance and mean arterial pressure were significantly lower with the addition of vatinoxan, showing dose-dependent reductions in peak values. Intramuscular pre-treatment with vatinoxan (200 µg kg\(^{-1}\)) 10 minutes prior to medetomidine (10 µg kg\(^{-1}\)) administration also prevented the medetomidine-induced increase in arterial pressure (Enouri et al. 2008).

Rolfe et al. (2012) investigated the cardiovascular effects following co-administration of 20 µg kg\(^{-1}\) medetomidine with 400 µg kg\(^{-1}\) of vatinoxan, given intramuscularly but as separate injections. They reported that the medetomidine-evoked dramatic increase in blood pressure was prevented by vatinoxan, although vascular pressures did increase slightly from baseline values. However, when Restitutti et al. (2017) studied the cardiovascular effects of vatinoxan (200, 400 and 600 µg kg\(^{-1}\)) co-administered intramuscularly in the same syringe with medetomidine (20 µg kg\(^{-1}\)), the initial hypertensive effect was not prevented. In fact, it appeared even more pronounced in the presence of the smallest dose of vatinoxan. The results indicated that the systemic antagonistic effects of vatinoxan appear slower than those of medetomidine (Restitutti et al. 2017). This finding was probably due to slower absorption of vatinoxan, resulting in delayed onset of vatinoxan’s effects compared to medetomidine, when the two drugs were given as a single intramuscular injection (Restitutti et al. 2017). Hence, the initial medetomidine evoked cardiovascular changes were not fully attenuated by vatinoxan. However, the duration of all cardiovascular effects of medetomidine were shortened dose-dependently by vatinoxan (Restitutti et al. 2017).

Pagel et al. (1998) reported that the later vasodepressor effects of dexmedetomidine were pronounced in the presence of vatinoxan, demonstrated by greater reductions in mean arterial and left ventricular systolic pressures. This outcome was expected since dexmedetomidine’s central effects are unaffected by the peripheral α2-adrenoceptor antagonist. Importantly, hypotension was not reported in any of the experiments in which vatinoxan was studied with either medetomidine or dexmedetomidine in dogs, irrespective of the dose or route of administration (Pagel et al. 1998, Enouri et al. 2008, Honkavaara et al. 2011, Rolfe et al. 2012, Restitutti et al. 2017). However, it was presumed that the vasodilatory effect attributed to vatinoxan combined with the central hypotensive effect of medetomidine, could be accentuated by other vasodilating drugs leading to hypotension.
Vatinoxan also attenuates dexmedetomidine-mediated increases in coronary vascular resistance and left ventricular systolic and end-diastolic pressures as well as the decreases in heart rate, cardiac output and myocardial contractility (Pagel et al. 1998). Those effects result from blockade of peripheral $\alpha_2$-adrenoceptors in venous and arterial vasculature (Szemeredi et al. 1989). Pagel et al. (1998) reported that vatinoxan dose-dependently attenuated or abolished dexmedetomidine-induced increases in left ventricular preload and afterload. Enouri et al. (2008) described more specifically that vatinoxan minimized the medetomidine-associated increase in pulmonary artery occlusion pressure and central venous pressure. Increases in these pressures are direct indices of right and left ventricular preload and influenced by venous return, capacitance and myocardial performance (Enouri et al. 2008).

Honkavaara et al. (2011) suggested that a dose ratio of 10 µg kg$^{-1}$ dexmedetomidine with 500 µg kg$^{-1}$ vatinoxan co-administered intravenously could be considered optimal. This was based on stable systemic haemodynamics with that dosing rate throughout the observation period in all dogs (Honkavaara et al. 2011). Rolfe et al. (2012) reported that administration route did not affect the cardiovascular outcome when the dose ratio was 1:20 of medetomidine:vatinoxan given concomitantly either intravenously or intramuscularly as separate injections.

In conclusion, although vatinoxan is not necessarily able to prevent or reverse all $\alpha_2$-adrenoceptor agonist-induced cardiovascular changes, it substantially reduces them and normalizes cardiovascular function by antagonizing $\alpha_2$-adrenoceptors that mediate vasoconstriction.

2.2.2.3 Ketamine

Injectable anaesthetic combinations are often used for minor surgeries in veterinary practice. Ketamine is an uncompetitive antagonist of N-methyl-D-aspartate receptors that in addition acts on a wide range of other targets responsible for various effects and uses. Ketamine’s effects on the central nervous system remain unclear, but the evidence indicates that ketamine-induced anaesthesia occurs via distribution of corticocortical information transfer in a frontal-to-parietal distribution (Lee et al. 2013). In addition to its anaesthetic effects, ketamine acts predominantly as a sympathomimetic agent, producing increases in arterial pressures, heart rate and cardiac output through stimulation of the central nervous system (Traber et al. 1968). Ketamine’s direct myocardial depressant effects may, however, be unmasked e.g. due to catecholamine depletion (Cook et al. 1991). Ketamine also causes direct relaxation of vascular smooth muscle, although, due to its sympathetically mediated vasoconstriction, systemic vascular resistance stays relatively stable (Diaz et al. 1976).

When ketamine (3 mg kg$^{-1}$) was coadministered with dexmedetomidine (15 µg kg$^{-1}$) and buprenorphine (40 µg kg$^{-1}$) in dogs, marked systemic hypertension and a transient increase in heart rate coupled with a decrease in cardiac output due to a reduction in stroke volume were observed (Ko et al. 2013). They stated that the addition of ketamine to the combination accentuated arterial hypoxaemia, increased pulmonary wedge pressures, and augmented systemic hypertension. Hence, the cardiopulmonary function was not improved by the addition of ketamine, although heart rate
was increased and severe bradycardia prevented (Ko et al. 2013). Haskins et al. (1986) noted that larger increases in arterial blood pressures were associated with simultaneous administration of an \( \alpha_2 \)-adrenoceptor agonist with ketamine compared to its intramuscular administration 5 minutes before intravenous ketamine. It was proposed that if ketamine is administrated with the \( \alpha_2 \)-adrenoceptor agonist such that their hypertensive effects coincide, the increase in arterial blood pressure might be severe (Haskins et al. 1986). However, when the drugs are administered in a sequence that allows the \( \alpha_2 \)-adrenoceptor agonist’s initial hypertensive effect to subside, there is no further pressor effect from subsequent ketamine administration. In view of that, Haskins et al. (1986) reported that ketamine did not change systemic blood pressure when administered to xylazine-premedicated dogs. Heart rate increased and returned to baseline values, whereas cardiac output increased only transiently and remained below pre-xylazine values (Haskins et al. 1986).

In summary the simultaneous intramuscular administration of ketamine and medetomidine is technically straightforward but produces undesirable cardiovascular effects. However, ketamine administration after medetomidine’s initial vasoconstrictive effects have waned might be a useful way to treat both the bradycardia and decrease in cardiac output. Since the effect is transient, ketamine might need to be administered repeatedly or as a continuous infusion to achieve more permanent increases.

2.2.2.4 Anaesthesia with isoflurane

Anaesthesia is often maintained with inhalant anaesthetics such as isoflurane in small animal practice. Isoflurane has been shown to dose-dependently reduce mean arterial pressure in dogs (Brahim and Thut 1984). The decrease in mean arterial blood pressure is mainly the result of a decrease in systemic vascular resistance (Mutoh et al. 1997). Pypendop and Verstegen (2000) as well as Ko et al. (2001a) reported that isoflurane attenuated the cardiovascular response to medetomidine; mean arterial pressure was lower and bradycardia less pronounced. Pypendop and Verstegen (2000) related their finding to that observed by Kenny et al. (1989), who found that isoflurane ameliorated the vasoconstrictive effect of the \( \alpha_2 \)-adrenoceptor agonist. Likewise, Kersten et al. (1993) reported that isoflurane attenuated both the intensity of vasoconstriction and decrease in cardiac output in dogs that had been given dexmedetomidine 30 µg kg\(^{-1}\) as oral premedication.

Dexmedetomidine reduces the requirement of isoflurane, as demonstrated by a significant decrease in the minimum alveolar concentration of the inhalant anaesthetic (Pascoe 2006). Since isoflurane produces negative inotropy combined with peripheral vasodilation, dexmedetomidine has been used as an anaesthetic adjunct to decrease the concentration-dependent cardiovascular depressant effects of isoflurane (Pascoe 2015). Cardiopulmonary changes were within clinically accepted normal ranges when dexmedetomidine with a loading dose of 0.5 µg kg\(^{-1}\) and a constant rate infusion of 0.5 µg kg\(^{-1}\) h\(^{-1}\) was administered during isoflurane anaesthesia in dogs. However, the achieved reduction in isoflurane concentration failed to improve cardiovascular function. And when the dose of the agonist was increased, cardiac output and oxygen delivery were markedly reduced compared with an equipotent dose of isoflurane alone (Pascoe 2015).
Medetomidine offsets the vasoactive effects of isoflurane because of its vasoconstrictive activity. Isoflurane is effective at lower end-tidal concentrations in the presence of medetomidine and this isoflurane-sparing effect might also mitigate some of isoflurane’s dose-dependent blood pressure lowering effects (Ewing et al. 1993). Kuusela et al. (2001) reported the effects of medetomidine given intravenously at 40 µg kg⁻¹ as premedication prior to propofol-isoflurane anaesthesia. Medetomidine’s peripheral vasoconstrictive effects predominated and isoflurane had only a minimal influence on heart rate and or arterial blood pressures. In contrast, with a much lower dose of medetomidine (0.4 µg kg⁻¹), mean arterial pressure decreased below reference range and was considered lower than that typically observed in dogs under a light plane of isoflurane anaesthesia. It was proposed that this hypotensive effect resulted from the combination of medetomidine’s central sympatholytic effects and blood pressure lowering action of isoflurane that, together, produced hypotension (Kuusela et al. 2001).

2.2.3 Prevention of prolonged sedation
Pypendop and Verstegen (1998) reported that the duration of sedation became longer with increasing doses of medetomidine. Kuusela et al. (2000) suggested that the degree of sedation does not increase when the intravenous dose of dexmedetomidine is increased above 10 µg kg⁻¹. Hence, prolongation of sedation can be prevented by using medetomidine doses ≤ 20 µg kg⁻¹ and selecting the minimum dose that provides the desired degree and duration of both sedation and antinociception. Co-administration with e.g. butorphanol might help reduce the medetomidine dose required for this approach (see section 2.2.1.1 for more information).

Vatinoxan reduces the duration of the sedative effect of medetomidine probably because of increased clearance and decreased concentration of plasma dexmedetomidine in the presence of this antagonist (Honkavaara et al. 2012, Bennett et al. 2016, Restitutti et al. 2017). Honkavaara et al. (2008) observed that vatinoxan did not affect the quality of dexmedetomidine-induced sedation. Restitutti et al. (2011) reported that vatinoxan did not reduce the dexmedetomidine-induced sedation to a degree that would be clinically relevant. Hence, co-administration of medetomidine with vatinoxan intravenously or intramuscularly decreases the likelihood of prolonged period of sedation without altering its initial intensity (Bennett et al. 2016, Restitutti et al. 2017). Unlike vatinoxan, atipamezole can be used to reverse the sedative effect of medetomidine since it distributes from the periphery to the central nervous system (see section 2.3 for more information).

Progressive decrease in body temperature following medetomidine administration has been reported, attributed to both muscle relaxation (that reduced shivering) and direct hypothalamic effects (Virtanen 1989, Pettifer and Dyson 1993, Pypendop et al. 1996, Pypendop and Verstegen 1998). Administering vatinoxan with medetomidine is likely to increase transcutaneous heat loss because of vasodilation and enhanced peripheral blood flow (Vainionpää et al. 2013). Hence, hypothermia (rectal temperature below 36.5 °C) is a possible complication during and after medetomidine-induced sedation especially in the presence of vatinoxan. Since hypothermia can prolong the action of drugs by slowing their metabolism, it is important to closely monitor and prevent this reduction in body temperature with appropriate management. Conservation of body heat would likely be beneficial in preventing prolonged sedation.
2.3 The use of atipamezole in dogs
Atipamezole is a highly selective and specific antagonist of central and peripheral \( \alpha_2 \)-adrenoceptors (\( \alpha_{2A}, \alpha_{2B} \) and \( \alpha_{2C} \) subtypes), producing potent antagonism of the actions of medetomidine (Virtanen 1989, Haapalinna et al. 1997). Atipamezole was developed for use in veterinary practice to shorten the recovery period after medetomidine-induced sedation, as well as when reversal was needed due to medetomidine-evoked cardiovascular complications (Virtanen 1989). Atipamezole antagonizes medetomidine-induced behavioural, neurochemical, cardiovascular, gastrointestinal and hypothermic changes in laboratory models (Scheinin et al. 1988, MacDonald et al. 1989, Savola 1989, Savola et al. 1989). When the pharmacokinetics of medetomidine were studied after intramuscular atipamezole administration, it was discovered that atipamezole increased medetomidine clearance and decreased its elimination half-life (Salonen et al. 1995).

2.3.1 Reversal of sedation
When laboratory beagles were given atipamezole intramuscularly 20 minutes after intramuscular medetomidine administration (dose rates of 2, 4, 6 and 10 times higher than the preceding medetomidine dose), deeply sedated dogs showed signs of arousal within 3–7 minutes and were able to walk within 4–12 minutes (Vainio and Vähä-Vahe 1990). Dose-related recovery was optimal when atipamezole was administered at 4 to 10 times the preceding dose of medetomidine, although drowsiness was reported in 41% of the dogs 30–60 minutes after atipamezole administration (Vainio and Vähä-Vahe 1990).

In a clinical trial, intramuscularly administered dose ratios of 2:1, 4:1, 5:1 and 6:1 atipamezole to medetomidine were investigated (Vähä-Vahe 1990). The median arousal time was 4 minutes and median walking time 10 minutes after administration of atipamezole. Sedation was successfully reversed with atipamezole 15 minutes after medetomidine administration. The overall effectiveness of reversal was rated as good or moderate in the majority of dogs, with the best ratings given for the 5:1 and 6:1 dose ratios. The incidence of adverse effects was minimal, but a few dogs vomited, some appeared overly alert or panted, whilst some dogs relapsed into drowsiness (Vähä-Vahe 1990). When atipamezole was administered intramuscularly to dogs after conclusion of a procedure performed under general anaesthesia, using five times the preceding intramuscular medetomidine dose, the dogs recovered smoothly and achieved sternal recumbency within approximately 15 minutes (Young et al. 1990).

2.3.2 Reversal of cardiovascular and respiratory depression
When atipamezole was administered to laboratory beagles 30 minutes after medetomidine, with a 5:1 to 10:1 dose ratios of atipamezole:medetomidine, it effectively increased heart rates (Vainio 1990). Heart rate increased from 40-46 (range) to 67-95 beats minute\(^{-1}\) within 10 minutes of atipamezole administration in a dose-dependent manner where the increase in heart rate was greater with the higher doses of atipamezole (Vainio 1990). However, Vainio and Vähä-Vahe (1990) noted that although heart rates increased after atipamezole administration, there was a secondary
decrease and that heart rate remained above pre-atipamezole levels only with 6:1 and 10:1 dose ratios. Vähä-Vahe (1990) reported that atipamezole reversed medetomidine-induced bradycardia, reaching 75% of the baseline values with a 6:1 dose ratio. When atipamezole was administered at a 4:1 dose ratio 40 min after medetomidine-midazolam sedation, Hayashi et al. (1994a) observed that systemic vascular resistance returned to baseline values and remained constant thereafter. Similarly, heart rate increased rapidly to near baseline values and remained significantly above pre-atipamezole values. Cardiac index increased to near baseline values within 20 min from atipamezole administration (Hayashi et al. 1994a). In a study by Pypendop et al. (1996) atipamezole was administered at a 2.5:1 dose ratio with medetomidine, 60 minutes following medetomidine-midazolam, medetomidine-midazolam-butorphanol or medetomidine-midazolam-buprenorphine treatment. The following cardiovascular changes were observed: systemic vascular resistance reverted to baseline and heart rate increased to values slightly below baseline immediately after atipamezole administration, but cardiac index remained significantly lower than baseline (Pypendop et al. 1996). In view of these studies, it seems that the capacity of atipamezole to fully reverse medetomidine-induced cardiovascular changes is deficient. Nevertheless, in all of the aforementioned studies conclusions were that atipamezole was effective in antagonizing the cardiovascular effects of medetomidine. More evidence is needed to draw this inference.

Atipamezole also abolished medetomidine-induced arrhythmias (Vainio 1990). Arterial blood pressure decreased significantly by 8-20 % immediately after atipamezole administration (Vainio 1990) This decrease was attributed to displacement of medetomidine from peripheral $\alpha_2$-adrenoceptors causing dilation of the vessels. However, blood pressure reverted to pre-reversal values 10 minutes following atipamezole administration (Vainio 1990). Similar decreases in mean arterial pressure after atipamezole administration were observed by Pypendop et al. (1996).

Both bradypnoea and the decrease in the arterial partial pressure of oxygen were reversed when atipamezole was used with 5-10 times the agonists’ dose (Vainio 1990). Hayashi et al. (1994a) considered the respiratory effects of medetomidine (20 µg kg$^{-1}$) and midazolam (0.3 mg kg$^{-1}$) to be minor, hence changes in the arterial partial pressure of oxygen were also minimal after atipamezole administration. Pypendop et al. (1996) reported that intramuscular administration of medetomidine did not affect arterial blood gas partial pressures when administered alone. However, respiratory depression with changes in arterial blood gas partial pressures was noticed when midazolam and an opioid were administered intravenously 20 minutes after medetomidine, and the changes could be reversed with atipamezole (Pypendop et al. 1996).

To conclude, atipamezole does not restore cardiovascular function into presedation level in medetomidine-sedated dogs. Hence, in clinical practice cardiovascular monitoring should be continued also after atipamezole administration to avoid complications during recover.
3 AIMS OF THE STUDY

The main objective of this thesis was to investigate the central and peripheral interactions between vatinoxan, atipamezole and ketamine in dogs sedated or premedicated with medetomidine.

The specific aims were:

1. To investigate the cardiovascular and sedative reversal efficacy of atipamezole in laboratory dogs treated with medetomidine with or without vatinoxan (Ⅰ)

2. To assess the effects of vatinoxan on the cardiovascular function, and quality of anaesthesia and recovery in laboratory dogs premedicated with medetomidine and butorphanol followed by ketamine, and subsequently reversed with atipamezole (Ⅱ)

3. To explore the clinical use of vatinoxan in healthy dogs of various breeds sedated for diagnostic imaging with medetomidine and butorphanol, and the need for reversal with atipamezole (Ⅲ)

4. To detect the influence of vatinoxan on plasma concentrations of other simultaneously used drugs in dogs (Ⅰ – Ⅲ)
4 MATERIALS AND METHODS

4.1 Animals
A total of eight purpose-bred laboratory beagle dogs (6 castrated males and 2 ovariohysterectomized females), considered healthy on the basis of history and comprehensive general examination, which included complete blood counts and routine serum biochemical analyses, participated in studies I and II. The dogs were 2 to 3 years of age and their mean (± standard deviation) body weight ranged from 13.3 ± 1.7 to 14.5 ± 1.5 kg during the experiments. The dogs were housed in groups in a kennel with daily maintenance and activities and they had already been familiarized with the personnel and research facilities before the studies. The dogs were fed commercial food and they had free access to water in the kennel. Food was withheld 12 hours prior to the experiments. After completion of the experimental day, the dog’s recovery was confirmed (heart rate and composite sedation score), food was offered, and one dose of meloxicam (see Table 2) was administered subcutaneously before transfer back to the kennel. The studies were approved by the Animal Experiment Board of Finland (ESAVI/7187/04.10.03/2012). All dogs have since been placed with private families.

Study III was performed at the Veterinary Teaching Hospital of University of Helsinki. For this study 56 client-owned dogs that required sedation for non-invasive diagnostic imaging, which consisted of radiographic imaging for the screening of canine genetic bone diseases and defects, were enrolled. The inclusion criteria were: American Society of Anesthesiologists physical status score of I or II; no breed-related contraindications for deep sedation (e.g. brachycephalic syndrome); no history or signs of known systemic disease; no administration of drugs affecting the central nervous system; body weight ≥ 5 kg, and age between 3 months and 10 years. Informed owner consent was obtained for each dog and the owners had the absolute right to withdraw their dogs from the study at any time. The breeds of the dogs that participated in the clinical trial are presented in Table 1. The study was approved by the Animal Experiment Board of Finland (ESAVI/6082/04.10.07/2016). All dogs were returned to their owners on the same day, once the procedure was completed.

Table 1. Breeds and numbers of dogs that participated in the clinical trial (III). Dog breeds that were given vatinoxan are highlighted with a gray background colour and the number of these dogs is indicated in parenthesis.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number (Presence)</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Staffordshire Terrier</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Australian Shepherd</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Basset Hound</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Bedlington Terrier</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Bohemian Shepherd</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Border Collie</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Cairn Terrier</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Cirneco Delletna</td>
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</tr>
<tr>
<td>Cocker Spaniel</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Entlebucher Sennenhund</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Flatcoated Retriever</td>
<td>3 (2)</td>
</tr>
<tr>
<td>German Shepherd</td>
<td>9 (5)</td>
</tr>
<tr>
<td>Giant Schnauzer</td>
<td>4 (1)</td>
</tr>
<tr>
<td>Golden Retriever</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Lapphund</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Lapponian Herder</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Miniature Schnauzer</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Mixed breed</td>
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</tr>
<tr>
<td>Mudi</td>
<td>2 (0)</td>
</tr>
<tr>
<td>Norwegian Elkhound</td>
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<tr>
<td>Podengo</td>
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<tr>
<td>Pyrenean Mastiff</td>
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<tr>
<td>Schweizer laufhund</td>
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</tr>
<tr>
<td>Whippet</td>
<td>3 (2)</td>
</tr>
</tbody>
</table>
4.2 Study design
Sample size calculations were based on data from previous studies undertaken by our research group. In earlier laboratory studies, eight dogs had been used in order to be able to detect the following differences: heart rate of 11 beats minute\(^{-1}\) (50 ± 10 versus 61 ± 10 beats minute\(^{-1}\)), cardiac output of 1.4 L minute\(^{-1}\) (4.0 ± 1.0 versus 5.4 ± 1.2 L minute\(^{-1}\)) and mean arterial pressure of 17 mm Hg (110 ± 10 mm Hg versus 93 ± 15 mm Hg), with a power of 80% and an α level of 0.05. For the clinical study, a power analysis, based on a difference of 20 beats minute\(^{-1}\) (40 ± 15 versus 60 ± 15 beats minute\(^{-1}\)) between groups in heart rate with a power of 80% and an α level of 0.05, suggested that 20 dogs per group was required.

In Studies I and II each dog was administered two treatments in a randomized (www.random.org), cross-over design, with a minimum 2-week washout period. Study III was a randomized (Microsoft Office Excel), complete block design, clinical trial and the randomization into groups was done in blocks for breed and body weight to ensure similar subject characteristics in either group.

Study treatments included either medetomidine and vatinoxan or medetomidine alone in all studies. Medetomidine was used as the control treatment. Subjective assessments (e.g. sedation) were made by observers masked to both the treatment and haemodynamic monitoring.

4.3 Instrumentation, drugs and dosages
In studies I and II, prior to each experiment, the dogs were instrumented under general anaesthesia with arterial and central venous catheters (see Table 2) used for cardiovascular monitoring, measurements and blood sampling. After instrumentation, the dogs were allowed to recover for a minimum of 60 minutes before baseline measurements.

In study III, instrumentation was minimally invasive since it was conducted with client-owned dogs. A catheter was inserted in either cephalic vein 10 minutes after treatment for blood sampling, and in case intravenous administration of drugs would have been needed.

Drugs, their doses and administration routes, materials and instruments used in studies I - III are displayed in Table 2. In studies I and II, the medetomidine-vatinoxan solution was prepared just prior to use, in a sterile vial containing 20 mg (I) or 25 mg (II) of vatinoxan powder, by adding 1 ml of medetomidine and 1 mL of 0.9% sterile saline solution into the vial and mixing it until the solution was clear by visual inspection. The final drug concentrations in the solution were 500 µg mL\(^{-1}\) of medetomidine and 10 (I) or 12.5 mg mL\(^{-1}\) (II) of vatinoxan. Hence, the dose ratios were 1:20 and 1:25, respectively. Other drugs used during the studies were commercially available.
### Table 2. Drugs, their doses and administration routes, materials and instruments used in studies I - III.

<table>
<thead>
<tr>
<th>Study</th>
<th>Timing</th>
<th>Drugs and dosages / materials / instruments used for the studies</th>
<th>Administration route / placing</th>
<th>Product information</th>
</tr>
</thead>
<tbody>
<tr>
<td>I, II</td>
<td>Instrumentation prior to the experiment</td>
<td>Propofol to effect</td>
<td>Intravenously</td>
<td>Verofet vet 10 mg mL⁻¹, Norbrook Laboratories, Monaghan, Ireland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhuffusor targeted at Et₅₀ of 3.5 %</td>
<td>Intravenously</td>
<td>Verfurane 1000 mg g⁻¹, Vitha S.A., Carros, France</td>
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<tr>
<td></td>
<td></td>
<td>Ringer’s acetate</td>
<td>Intravenously</td>
<td>Ringer-Acetate Baxter Vials, Baxter, Helsinki, Finland</td>
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<tr>
<td></td>
<td></td>
<td>Lidocaine 0.25 mL</td>
<td>Subcutaneously</td>
<td>Lidocaine 20 mg mL⁻¹, Orion Pharma, Espoo, Finland</td>
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<tr>
<td></td>
<td></td>
<td>Arterial catheter</td>
<td>Arterio femoralis</td>
<td>Artenufl II B Braun Melsungen AG, Berlin, Germany</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Central venous catheter</td>
<td>Vena jugularis</td>
<td>CV 12702, Arrow International, PA, USA</td>
</tr>
<tr>
<td>During the experiment</td>
<td>Anaesthesia monitor</td>
<td>Connected to the dog</td>
<td>SAS, GE Healthcare, Helsinki, Finland</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pressure transducers</td>
<td>Arterial and venous lines</td>
<td>Gabathir PMSET, Benton Dickinson, Sandy, USA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LUCO monitor</td>
<td>Arterial line</td>
<td>LUCO Plus hemodynamic monitor, LUCO Ltd, London, England</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lithium chloride solution</td>
<td>Vena jugularis</td>
<td>Lithium chloride (0.15 mmol mL⁻¹) solution for injection, LUCO Ltd, London, England</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heparinized syringes</td>
<td>Arterial blood samples</td>
<td>Pico 50, Radiometer Medical Aps, Copenhagen, Denmark</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Blood gas analyser</td>
<td>Arterial blood samples</td>
<td>ABL 855, Radiometer Medical Aps, Copenhagen, Denmark</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>-30 min</td>
<td>Medetomidine 20 µg kg⁻¹</td>
<td>Intravenously into gastrocnemius muscles</td>
<td>Dierlene 1 mg mL⁻¹, Laboratoires SVVA S.A.U., León, Spain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vatinoxan 400 µg kg⁻¹</td>
<td>Intravenously over 2 min</td>
<td>Vetaqin 0.4 mg mL⁻¹, Vetaqin Oy, Salo, Finland</td>
</tr>
<tr>
<td>0 min</td>
<td></td>
<td>Atipamezole 100 µg kg⁻¹</td>
<td>Intravenously into gastrocnemius muscles</td>
<td>Altane 5 mg mL⁻¹, Laboratoires SVVA S.A.U., León, Spain</td>
</tr>
<tr>
<td>After study</td>
<td>Meloxicam 0.2 mg kg⁻¹</td>
<td>Subcutaneously</td>
<td>Metacam 5 mg mL⁻¹, Boehringer Ingelheim Vetmedica GmbH, Rhein, Germany</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>-20 min</td>
<td>Medetomidine 20 µg kg⁻¹</td>
<td>Intravenously into gastrocnemius muscles</td>
<td>Dierlene 1 mg mL⁻¹, Laboratoires SVVA S.A.U., León, Spain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vatinoxan 500 µg kg⁻¹</td>
<td>Intravenously over 2 min</td>
<td>Vetaqin 0.4 mg mL⁻¹, Vetaqin Oy, Salo, Finland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Butorphanol 100 µg kg⁻¹ added to same syringe</td>
<td>Intravenously over 2 min</td>
<td>Torpudur vet 10 mg mL⁻¹, Richter Pharma AG, Weis, Austria</td>
</tr>
<tr>
<td>0 min</td>
<td></td>
<td>Ketamine 4 mg kg⁻¹</td>
<td>Intravenously over 2 min</td>
<td>Ketaminol 50 mg mL⁻¹, Intervet International B.V., Bozeman, The Netherlands</td>
</tr>
<tr>
<td>60 min</td>
<td></td>
<td>Atipamezole 100 µg kg⁻¹</td>
<td>Intravenously into gastrocnemius muscles</td>
<td>Altane 5 mg mL⁻¹, Laboratoires SVVA S.A.U., León, Spain</td>
</tr>
<tr>
<td>After study</td>
<td>Meloxicam 0.2 mg kg⁻¹</td>
<td>Subcutaneously</td>
<td>Metacam 5 mg mL⁻¹, Boehringer Ingelheim Vetmedica GmbH, Rhein, Germany</td>
<td></td>
</tr>
</tbody>
</table>

### 4.4 Measurements and assessments

Time points of measurements and assessments are presented in Table 3.

### Table 3. Time points of measurements and assessments in studies I - III. BL refers to baseline. MED refers to medetomidine treatment and MEDBUT to medetomidine-butorphanol treatment, with or without vatinoxan. KET refers to ketamine and ATI to atipamezole administration, respectively. In study III atipamezole was administered only to dogs unable to stand or walk after completion of procedure.

<table>
<thead>
<tr>
<th>Study</th>
<th>Timing</th>
<th>Measurements</th>
<th>BL</th>
<th>-20</th>
<th>-10</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>210</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
<td>Heart rate</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
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<td>☑️</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Respiratory rate</td>
<td>☑️</td>
<td>☑️</td>
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<td>☑️</td>
<td>☑️</td>
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<td>☑️</td>
<td>☑️</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arterial blood pressures</td>
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<td>☑️</td>
<td>☑️</td>
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<td>☑️</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Cardiac output</td>
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<td>☑️</td>
<td>☑️</td>
<td>☑️</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Arterial blood sample</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Venous blood sample</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Sedation</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Rectal temperature</td>
<td>☑️</td>
<td>☑️</td>
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</tr>
</tbody>
</table>
### Study II

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Time point (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
</tr>
<tr>
<td>Heart rate</td>
<td>✔</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>✔</td>
</tr>
<tr>
<td>Arterial blood pressures</td>
<td>✔</td>
</tr>
<tr>
<td>Cardiac output</td>
<td>✔</td>
</tr>
<tr>
<td>Arterial blood sample</td>
<td>✔</td>
</tr>
<tr>
<td>Venous blood sample</td>
<td>✔</td>
</tr>
<tr>
<td>Sedation</td>
<td>✔</td>
</tr>
<tr>
<td>Rectal temperature</td>
<td>✔</td>
</tr>
<tr>
<td>Recovery</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Study III

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Time point (minutes)</th>
<th>At 10 minutes intervals</th>
<th>At 30 minutes intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Heart rate</td>
<td>✔</td>
<td>M</td>
<td>✔</td>
</tr>
<tr>
<td>Respiratory rate</td>
<td>✔</td>
<td>E</td>
<td>✔</td>
</tr>
<tr>
<td>Sedation</td>
<td>✔</td>
<td>D</td>
<td>✔</td>
</tr>
<tr>
<td>Mucous membranes</td>
<td>✔</td>
<td>B</td>
<td>✔</td>
</tr>
<tr>
<td>Venous blood sample</td>
<td>✔</td>
<td>T</td>
<td>✔</td>
</tr>
<tr>
<td>Rectal temperature</td>
<td>✔</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.4.1 Cardiopulmonary measurements
In studies I and II, at the start of an experiment, the dogs were minimally restrained in sternal recumbency on the examination table, which was covered with an isolating mattress and electrical heating pad. The arterial and central venous catheters were connected to pressure transducers (see Table 2 for details), that had been tested against a mercury manometer and zeroed to atmospheric pressure at the level of manubrium just prior to the experiment. Direct arterial and venous blood pressures and continuous lead II electrocardiography were monitored throughout the studies. Heart rates were counted ( I ) or verified ( II ) by auscultation, and respiratory rates were measured by counting chest movements in one minute. Cardiac output was measured with the lithium dilution method as described by Mason et al. (2001) with a standard dose of 0.075 mmol of lithium chloride. Initial standard values of 10 g dL\(^{-1}\) for haemoglobin and 140 mmol L\(^{-1}\) for sodium were later corrected with actual values measured from simultaneously drawn arterial blood gas samples. Arterial blood gas samples were anaerobically collected into heparinized syringes prior to each cardiac output measurements, stored in iced water and analyzed within 15 minutes. Temperature-corrected partial pressures of oxygen and carbon dioxide, pH, and arterial lactate concentrations were recorded. Cardiac index, stroke volume, rate pressure product and systemic vascular resistance index were calculated by using standard equations (Haskins et al. 2005).

In study III heart rates were auscultated and respiratory rates assessed by counting of thoracic movements. Measurement of non-invasive blood pressures was also attempted but results are not presented.

4.4.2 Body temperature
All studies were performed in room temperature. The dogs’ body temperature was supported during the studies with external heating including blankets and heating pads to maintain rectal temperature above 36.5 °C.

4.4.3 Clinical assessment of sedation, anaesthesia induction and tracheal intubation
Scoring systems used to assess sedation ( I , II ), induction of anaesthesia and tracheal intubation ( II ) are shown in Tables 4 and 5. Composite sedation scores were modified from Kuusela et al. (2001) ( I , II ). In study III sedation was determined using a visual analogue sedation scale between 0 to 100, where 0 represents no signs of sedation and 100 demonstrates a dog in lateral recumbency, unresponsive to a loud hand clap. Induction and intubation scores in study II were assessed according to Casoni et al. (2015).
4.4.4 Quality of recovery and owner questionnaire study

The quality of recovery was assessed after anaesthesia with a simple descriptive score in study II (Table 5). Recovery from sedation was assessed with the same composite sedation score (I, II) and visual analogue scale (III) as sedation. Adverse effects were recorded.

In the clinical study (III) a phone interview with the dogs’ owners’ was conducted the day after the procedure. Owners were asked about lethargy or any unusual behaviour after discharge and possible changes in appetite and defaecation.

Table 4. Composite sedation scores used in studies I and II.

<table>
<thead>
<tr>
<th>Position</th>
<th>Resistance to positioning in lateral recumbency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Standing</td>
<td>0 Normal</td>
</tr>
<tr>
<td>1 Standing but staggers</td>
<td>1 Turns back to sternal position</td>
</tr>
<tr>
<td>2 Sternal head up</td>
<td>2 Some resistance but stays in lateral recumbency</td>
</tr>
<tr>
<td>3 Sternal head down</td>
<td>3 No resistance or the position is already lateral</td>
</tr>
<tr>
<td>4 Lateral head down</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Palpebral reflex</th>
<th>Position of the eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Normal</td>
<td>0 Middle</td>
</tr>
<tr>
<td>1 Slightly reduced</td>
<td>2 Turned down</td>
</tr>
<tr>
<td>2 Weak</td>
<td></td>
</tr>
<tr>
<td>3 Absent</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Jaw and tongue relaxation</th>
<th>General appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Normal, opens the jaws but resist manipulation of the tongue</td>
<td>0 Normal</td>
</tr>
<tr>
<td>1 Bites jaws together</td>
<td>1 Slightly tired, head drooping</td>
</tr>
<tr>
<td>2 Opens the jaws but strong resistance when tongue is pulled</td>
<td>2 Mild sedation, reacts clearly to surroundings</td>
</tr>
<tr>
<td>3 Slight resistance when tongue is pulled</td>
<td>3 Moderate sedation, reacts slightly to surroundings</td>
</tr>
<tr>
<td>4 No resistance</td>
<td>4 Deep sedation, no reaction to surroundings</td>
</tr>
</tbody>
</table>

Total score maximum of 20
Table 5. Intubation, induction and recovery scores used in study II.

<table>
<thead>
<tr>
<th>Induction score</th>
<th>Intubation score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Ideal: smooth, uneventful induction</td>
<td>1 Smooth intubation without reaction</td>
</tr>
<tr>
<td>2 Good: mild twitching or excitement, head movements</td>
<td>2 Mild coughing during or immediately after intubation</td>
</tr>
<tr>
<td>3 Unsatisfactory: pronounced twitching or excitement, uncoordinated movements or paddling</td>
<td>3 Pronounced coughing during or immediately after intubation</td>
</tr>
<tr>
<td>4 Induction not reached</td>
<td>4 Swallowing, gagging, head movements during or immediately after intubation</td>
</tr>
<tr>
<td></td>
<td>5 Failed attempt to intubate</td>
</tr>
</tbody>
</table>

Early recovery score (after intubation)                                             Late recovery score (120 minutes after ketamine)

<table>
<thead>
<tr>
<th>Early recovery score (after intubation)</th>
<th>Late recovery score (120 minutes after ketamine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Easy transition to conscious, holds head up, coordinated movements</td>
<td>1 Alert and responsive, able to stand up and walk, coordinated movements, no signs of ataxia, depression or drowsiness</td>
</tr>
<tr>
<td>2 Fairly easy transition, holds head up, no body movement attempted</td>
<td>2 Slightly sedated and slow to react, able to stand up and walk, mild signs of ataxia, depression or drowsiness</td>
</tr>
<tr>
<td>3 Restless, uncoordinated movements or vocalizing</td>
<td>3 Sedated, able to stand but reluctant to walk, ataxia, depression or drowsiness</td>
</tr>
<tr>
<td>4 Uncoordinated whole body movements, needs restraint</td>
<td>4 Very sedated, unable to stand up and walk, unresponsive to stimuli, depressed, drowsy and sleepy</td>
</tr>
</tbody>
</table>

4.5 Concentrations of drugs in plasma

Venous blood samples for the analysis of plasma drug concentrations were collected at selected time points presented in Table 3. Venous samples were taken into EDTA tubes and centrifuged at 2300 g for 15 minutes. Plasma was separated into cryotubes and stored at ≤ -20 °C until analyzed using a high-performance liquid chromatography-tandem mass spectrometry method. More detailed descriptions of the analyses of plasma drug concentrations are found in the respective original papers from studies I – III.
4.6 Statistical analyses
For all statistical analyses, a p-value of < 0.05 was considered significant.

4.6.1 Studies I and II
Normality assumptions were checked with the Kolmogorov-Smirnov’s test. In case criteria for normal data distribution were not met, either logarithmic or inversed transformations were used. However, in the Figures of Section 5 (Results), descriptive data are presented in linear scale. Differences in several responses with and between treatments were evaluated with repeated measures analysis of covariance models. For between comparisons, actual values were used as response in the model, and for within treatment comparisons change from baseline was used. The models consisted of the main effects of treatment and timepoint, 2-way-interaction of treatment and time point, and a baseline covariate as fixed effects, and the main effect of dog, the two-way-interactions of dog and time point and dog and treatments as random effects. Estimates of treatment differences were calculated over time and by time point from the fitted models. Composite sedation scores and continuous variables measured only once were analysed with the Wilcoxon’s signed rank sum test or paired t-tests, respectively. Differences in plasma drug concentrations and areas under the curve were compared with paired, 2-tailed t-tests. SAS for Windows, version 9.3 (SAS Institute) was used for all statistical analyses.

4.6.2 Study III
Normality assumptions were checked with the Shapiro–Wilk test. Continuous parametric data was evaluated by general linear model analysis of covariance, where time was a within-subject factor and treatment was a between subject factor. Plasma drug concentrations and ‘head down’ time were compared with independent samples t-tests. The Mann–Whitney U test was used for visual analogue scales to compare treatments at each time point, and the related samples Friedman’s two-way analysis of covariance by ranks was used to compare each treatment against baseline. The Holm-Bonferroni post-hoc correction was used for multiple comparisons between treatments and within treatments. The two-sided Fischer’s exact test was used to compare the need for additional medetomidine or atipamezole, presence of loose faeces, lethargy and decreased appetite between the treatments. Analysis were computed using SPSS Software (IBM SPSS Statistics, version 24).
5 RESULTS

5.1 Cardiovascular effects

5.1.1 Systemic vascular resistance index

Data on systemic vascular resistance indices from studies I and II are presented in Figures 1 and 2. Systemic vascular resistance index increased significantly from baseline after medetomidine administration, but also when vatinoxan was co-administered. However, the magnitude of the increase was less in the presence of vatinoxan.

In study I, systemic vascular resistance index decreased after atipamezole administration with both treatments but remained higher than baseline without vatinoxan.

In study II, systemic vascular resistance index decreased after ketamine with both treatments but remained elevated from baseline without vatinoxan until atipamezole administration. Whereas with vatinoxan, systemic vascular resistance index after ketamine remained significantly lower than baseline throughout the observation period.

![Figure 1](image)

*Figure 1. Mean values ± standard deviation of systemic vascular resistance indices over time for dogs treated with intramuscular medetomidine (□) or medetomidine-vatinoxan (●). Atipamezole was administered intramuscularly 30 minutes after treatment at time point 0 minutes (▲). *Significant difference between treatments (p < 0.05) †Significantly different from baseline*
Figure 2. Mean values ± standard deviation of systemic vascular resistance indices over time for dogs treated with intramuscular butorphanol and medetomidine (□) or medetomidine-vatinoxan (●). Ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (❶) and atipamezole was administered intramuscularly 60 minutes after ketamine (❷). *Significant difference between treatments (p < 0.05) †Significantly different from baseline ‡Significantly different from before ketamine administration §Significantly different from before atipamezole administration.

5.1.2 Mean arterial pressure

Data on mean arterial pressures from studies I and II are presented in Figure 3 and 4. Mean arterial pressure increased from baseline after medetomidine administration, but co-administration of vatinoxan prevented the increase (I, II). In study I, atipamezole also decreased mean arterial pressure significantly soon after its administration, but hypotension (mean arterial pressure < 60 mmHg) was not observed regardless of the presence or absence of vatinoxan. Following induction of anaesthesia with ketamine in study II, arterial blood pressure decreased with both treatments and mild hypotension (values of 57–59 mmHg) was observed 40 and 55 minutes after ketamine administration in two out of eight dogs that were given vatinoxan. Atipamezole administered 60 minutes after ketamine, i.e. 80 minutes after treatment, increased mean arterial pressures significantly within the treatment including vatinoxan.
**Figure 3.** Mean values ± standard deviation of mean arterial pressures over time for dogs treated with intramuscular medetomidine (□) or medetomidine-vatinoxan (●). Atipamezole was administered intramuscularly 30 minutes after treatment at time point 0 minutes (▲). *Significant difference between treatments (p < 0.05) †Significantly different from baseline

**Figure 4.** Mean values ± standard deviation of mean arterial pressures over time for dogs treated with intramuscular butorphanol and medetomidine (□) or medetomidine-vatinoxan (●). Ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (▲) and atipamezole was administered intramuscularly 60 minutes after ketamine (▼). *Significant difference between treatments (p < 0.05) †Significantly different from baseline ‡Significantly different from before ketamine administration §Significantly different from before atipamezole administration
5.1.3 Heart rate

Heart rates from studies I – III are presented in Figures 5, 6 and 7. Medetomidine-evoked bradycardia (heart rate < 60 beats minute⁻¹) was detected in all studies. Co-administration of vatinoxan attenuated but did not fully prevent the decrease in heart rate (I – III). In study II heart rate increased transiently after ketamine administration. However, without vatinoxan heart rate decreased again below baseline 15 minutes after ketamine administration and remained lower than with vatinoxan until the end of the 120-minute observation period. After atipamezole administration in studies I and II, heart rate remained below baseline values throughout the observation period without vatinoxan. Whereas with vatinoxan, heart rate returned to or above baseline.

Figure 5. Mean values ± standard deviation of heart rates over time for dogs treated with intramuscular medetomidine (□) or medetomidine-vatinoxan (●). Atipamezole was administered intramuscularly 30 minutes after treatment at time point 0 minutes (▲). *Significant difference between treatments (p < 0.05) †Significantly different from baseline
**Figure 6.** Mean values ± standard deviation of heart rates over time for dogs treated with intramuscular butorphanol and medetomidine (□) or medetomidine-vatinoxan (●). Ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (1) and atipamezole was administered intramuscularly 60 minutes after ketamine (2). *Significant difference between treatments (p < 0.05) †Significantly different from baseline ‡Significantly different from before ketamine administration §Significantly different from before atipamezole administration

**Figure 7.** Mean values ± standard deviation of heart rates over time for dogs treated with intramuscular medetomidine (□) or medetomidine-vatinoxan (●) with butorphanol. *Significant difference between treatments (p < 0.05)

### 5.1.4 Cardiac index

Cardiac index results from studies I and II are presented in Figures 8 and 9. Cardiac index decreased significantly from baseline with both treatments but remained significantly higher when vatinoxan was co-administered (I, II). In study I, atipamezole failed to increase cardiac index without the presence of vatinoxan. Although cardiac index increased in study II after ketamine administration with both treatments, it remained below baseline when vatinoxan was not included.
Results

**Figure 8.** Mean values ± standard deviation of cardiac index over time for dogs treated with intramuscular medetomidine (□) or medetomidine-vatinoxan (●). Atipamezole was administered intramuscularly 30 minutes after treatment at time point 0 minutes (▲). *Significant difference between treatments (p < 0.05) †Significantly different from baseline

**Figure 9.** Mean values ± standard deviation of cardiac index over time for dogs treated with intramuscular butorphanol and medetomidine (□) or medetomidine-vatinoxan (●). Ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (❶) and atipamezole was administered intramuscularly 60 minutes after ketamine (❷). *Significant difference between treatments (p < 0.05) †Significantly different from baseline ‡Significantly different from before ketamine administration §Significantly different from before atipamezole administration
5.1.5 Associations between cardiovascular variables and changes

Cardiovascular data from study II are combined in Figures 10-12 to illustrate the association between separate haemodynamic changes. When medetomidine was administered without vatinoxan, the increase in systemic vascular resistance index was not associated with a proportionate increase in mean arterial blood pressure due to a larger decrease in cardiac index (II A, C). The decrease in mean arterial pressure after ketamine administration was related to a decline in systemic vascular resistance index in the presence of vatinoxan (II B). Heart rate and cardiac index appeared to be strongly associated within both treatments (II C, D). Conversely, cardiac index decreased when systemic vascular resistance index increased after both treatments (II E, F). However, cardiac index was higher and systemic vascular resistance index lower with treatment including vatinoxan, whereas in the absence of vatinoxan cardiac index remained lower and systemic vascular resistance index higher throughout the observation period (II E, F). The last two Figures demonstrate that mean arterial pressure was better maintained without vatinoxan because systemic vascular resistance index was higher, whereas cardiac index remained low, when compared to the treatment with vatinoxan.

After ketamine administration, systemic vascular resistance index and mean arterial pressure decreased, and cardiac index and heart rate increased with both treatments (II A-D). Once atipamezole was administered, systemic vascular resistance index decreased with both treatments and the increase in mean arterial blood pressure was associated with the increase in cardiac index rather than with heart rate (II A-F).

![Figure 10](image1.png)

**Figure 10.** Cardiovascular data from study II combined to illustrate the associations between systemic vascular resistance index (left side y-axis, dotted line) and mean arterial pressure (right side y-axis, closed line) when ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (Ⅰ) and atipamezole was administered intramuscularly 60 minutes after ketamine (Ⅱ).

Mean values ± standard deviation of systemic vascular resistance indices (dynes seconds cm⁻⁵ m⁻²) and mean arterial pressures (mmHg) over time (minutes) II A) in dogs treated with intramuscular medetomidine-butorphanol (□), and II B) in dogs treated with intramuscular medetomidine-vatinoxan-butorphanol (●).
Figure 11. Cardiovascular data from study II combined to illustrate the associations between heart rate (left side y-axis, dotted line) and cardiac index (right side y-axis, closed line) when ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (1) and atipamezole was administered intramuscularly 60 minutes after ketamine (2).

Mean values ± standard deviation of heart rates (beats minute\(^{-1}\)) and cardiac indices (L minute\(^{-1}\) m\(^{-2}\)) over time (minutes) II C in dogs treated with intramuscular medetomidine-butorphanol (□), and II D in dogs treated with intramuscular medetomidine-vatinoxan-butorphanol (●).

Figure 12. Cardiovascular data from study II combined to illustrate the associations between systemic vascular resistance index (left side y-axis, dotted line) and cardiac index (right side y-axis, closed line) when ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (1) and atipamezole was administered intramuscularly 60 minutes after ketamine (2).

Mean values ± standard deviation of systemic vascular resistance indices (dynes seconds cm\(^{-5}\) m\(^{-2}\)) and cardiac indices (L minute\(^{-1}\) m\(^{-2}\)) over time (minutes) II E in dogs treated with intramuscular medetomidine-butorphanol (□), and II F in dogs treated with intramuscular medetomidine-vatinoxan-butorphanol (●).

5.2 Pulmonary effects
Significant decreases in respiratory rates from baseline were observed after treatment administration in studies I – III, and there were no significant differences between treatments in any studies. In study III the lowest respiratory rates were detected at 20 – 30 minutes, with median values of 8 and 10 breaths minute\(^{-1}\) with and without vatinoxan, respectively. When butorphanol was co-administered with medetomidine and vatinoxan in study II, the decrease in arterial partial pressure of oxygen and increase in arterial partial pressure of carbon dioxide was significantly greater 10 minutes after treatment administration in the presence of vatinoxan. When butorphanol
was not included in the treatment (I), the aforementioned effect was not observed. In study I respiratory rates remained lowered throughout the observation period even after atipamezole administration but arterial partial pressures of both oxygen and carbon dioxide remained unchanged, with only sporadic differences from the baseline. In study II assisted ventilation was needed after ketamine due to apnoea in six out of eight dogs with both treatments.

5.3 Arterial pH and lactate
Although statistically significant changes in arterial pH and lactate concentrations were detected between treatments in studies I and II, values were within reference ranges (Haskins et al. 2005). During the 60-minute period after atipamezole administration in study I, arterial pH was significantly lower and lactate significantly higher in the absence of vatinoxan. In study II significant differences between treatments were observed 10 minutes after treatment administration with a larger decrease in pH and increase in lactate concentration without vatinoxan.

5.4 Sedation, hypnosis, induction and intubation
Sedation variables from study III are presented in Table 6. The co-administration of vatinoxan did not impair the quality of medetomidine-induced sedation or ketamine-evoked hypnosis, but the duration of both was shortened (I – III). When the initial level of sedation was compared in the clinical study, the onset of sedation appeared faster and sedation was deeper for the first 30 minutes when vatinoxan was included in the treatment (III). In laboratory studies no differences were detected in the initial sedation when composite sedation scores were compared between treatments (I, II).

Induction and intubation scores after ketamine did not differ between treatments (II). Induction of anaesthesia with ketamine was assessed as ‘ideal’ in all dogs with both treatments. Endotracheal intubation was mostly smooth, but mild coughing was recorded after intubation in three of the eight dogs that given vatinoxan and two of the eight dogs without vatinoxan (II).

The use of vatinoxan shortened the duration of anaesthesia in study II demonstrated by earlier intolerance to the endotracheal tube. The dogs tolerated being intubated for 52 ± 14 minutes with vatinoxan compared to 62 ± 14 minutes without it when atipamezole was administered at 60 minutes. Hence, in the presence of vatinoxan six of the eight dogs were extubated before atipamezole administration compared to one of the eight dogs without vatinoxan. Also, the composite sedation scores were significantly lower with vatinoxan at 55 and 60 minutes after ketamine administration.
Table 6. Results for 56 dogs sedated for diagnostic radiography with medetomidine-butorphanol or medetomidine-vatinoxan-butorphanol medication.

<table>
<thead>
<tr>
<th>Study III</th>
<th>Without vatinoxan (n = 29)</th>
<th>With vatinoxan (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head down mean ± standard deviation</td>
<td>297.5 ± 74.3 seconds</td>
<td>241.4 ± 77.8 seconds*</td>
</tr>
<tr>
<td>Duration of required sedation mean ± standard deviation (range)</td>
<td>66 ± 14.5 minutes (30 – 94 minutes)</td>
<td>66 ± 19 minutes (42 – 111 minutes)</td>
</tr>
<tr>
<td>Number of dogs requiring additional medetomidine (time)</td>
<td>1 (30 minutes)</td>
<td>7 (53 ± 10 minutes)</td>
</tr>
<tr>
<td>Number of dogs given atipamezole after procedure</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>Area under the curve for visual analogue sedation values until 30 minutes from drug administration</td>
<td>2445 (1855 – 2585)</td>
<td>2555 (1868 – 2750)*</td>
</tr>
</tbody>
</table>

*Significant difference between treatments (p < 0.05)
5.5 Recovery

5.5.1 Recovery from anaesthesia
In study II, the majority of recoveries from anaesthesia were uneventful with both treatments in the study, although one dog given vatinoxan acted restlessly during the early recovery period. No differences were observed between treatments; six dogs were alert and responsive and two dogs were slightly sedated or slow to react at the last observation point. Adverse effects including nausea, expressed as drooling, swallowing or lack of appetite, was recorded after atipamezole administration in three out of eight dogs when the treatment included vatinoxan and two out of eight dogs without vatinoxan. Furthermore, defaecation was observed in one dog without vatinoxan and tenesmus with mucoid faeces in one dog given vatinoxan.

5.5.2 Recovery from sedation and owner questionnaire study
Vatinoxan shortened the duration of sedation, and atipamezole successfully reversed sedation with both treatments. The presence of vatinoxan reduced the need for atipamezole administration in the clinical trial (Ⅲ). In study I atipamezole was administered 30 minutes after treatment administration, and composite sedation scores declined initially with both treatments. However, composite sedation scores started to increase again 30 minutes after atipamezole administration when vatinoxan was not part of the treatment. Significantly higher composite scores were detected 60 and 90 minutes following atipamezole administration with medetomidine when compared to medetomidine-vatinoxan treatment. With vatinoxan, sedation scores decreased steadily over time. Altogether, however, recoveries were uneventful after both treatments in studies I and III. The most commonly reported side effects in the owner questionnaires with both treatments were lethargy and loose faeces that resolved without medical care (Ⅲ). Lethargy was observed in 90% of the dogs without vatinoxan and 63% with vatinoxan during the evening after the study. Loose faeces were reported in 33% and 24% of the dogs with and without vatinoxan, respectively.

5.6 Concentrations of drugs in plasma
Concentrations of drugs in plasma from studies I and II are presented in Figures 13 and 14. Plasma dexmedetomidine and butorphanol concentrations from study III and areas under the plasma drug concentration-time curves of dexmedetomidine, atipamezole and ketamine from studies I and II are presented in Table 7. In study III the plasma concentrations of medetomidine and butorphanol were significantly higher in the presence of vatinoxan when measured in venous blood samples taken between 11-18 minutes after treatment administration. In study I dexmedetomidine concentrations peaked before atipamezole administration when vatinoxan was included in the treatment. Without vatinoxan, the highest mean concentration of dexmedetomidine was measured at 5 minutes after atipamezole administration. However, the concentrations of co-administered drugs also decreased more rapidly with than without vatinoxan (I, II). In study I the reduced exposure of other drugs in the presence of vatinoxan was demonstrated by significantly smaller areas under the plasma drug concentration-time curves of dexmedetomidine and atipamezole. In study II the aforementioned effect was observed with ketamine.
Figure 13. Mean values ± standard deviation of dexmedetomidine, atipamezole and vatinoxan plasma concentrations over time for dogs treated with intramuscular medetomidine (□) or medetomidine-vatinoxan (●). Atipamezole was administered intramuscularly 30 minutes after treatment at time point 0 minutes (▲). *Significant difference between treatments (p < 0.05)
**Results**

**Figure 14.** Mean values ± standard deviation of dexmedetomidine, butorphanol, ketamine and vatinoxan plasma concentrations over time for dogs treated with intramuscular medetomidine-butorphanol (□) or medetomidine-vatinoxan-butorphanol (●). Ketamine was administered intravenously 20 minutes after treatment at time point 0 minutes (❶) and atipamezole was administered intramuscularly 60 minutes after ketamine (❷). *Significant difference between treatments (p < 0.05)*

**Table 7.** Mean values ± standard deviation of medetomidine and butorphanol concentrations in plasma (Ⅲ) and areas under the plasma drug concentration-time curves of dexmedetomidine (Ⅰ), atipamezole (Ⅰ) and ketamine (Ⅱ).

<table>
<thead>
<tr>
<th>Study</th>
<th>Time point</th>
<th>Measurement</th>
<th>Drug</th>
<th>Without vatinoxan</th>
<th>With vatinoxan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ⅲ</td>
<td>11 – 18 minutes after treatment administration</td>
<td>Plasma concentration</td>
<td>Medetomidine</td>
<td>9 ± 3 ng mL⁻¹</td>
<td>16 ± 7 ng mL⁻¹*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Butorphanol</td>
<td>14 ± 4 ng mL⁻¹</td>
<td>35 ± 15 ng mL⁻¹*</td>
</tr>
<tr>
<td>Ⅰ</td>
<td>-10 – 210 minutes from atipamezole administration</td>
<td>Area under the plasma drug concentration-time curve</td>
<td>Dexmedetomidine</td>
<td>208 ± 31 min ng mL⁻¹</td>
<td>163 ± 25 min ng mL⁻¹*</td>
</tr>
<tr>
<td>Ⅰ</td>
<td>5 – 210 minutes from atipamezole administration</td>
<td>Atipamezole</td>
<td>3966 ± 798 min ng mL⁻¹</td>
<td>2655 ± 364 min ng mL⁻¹*</td>
<td></td>
</tr>
<tr>
<td>Ⅱ</td>
<td>10 – 70 minutes after ketamine administration</td>
<td>Ketamine</td>
<td>63 600 ± 14 700 min ng mL⁻¹</td>
<td>36 700 ± 5200 min ng mL⁻¹*</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference between treatments (p < 0.05)
6 DISCUSSION

6.1 Reduction of medetomidine’s undesirable cardiovascular effects with vatinoxan during sedation, ketamine anaesthesia and atipamezole reversal

Overall, vatinoxan attenuated the decrease in heart rate and cardiac index associated with medetomidine administration by reducing systemic vascular resistance index (I, II). However, it was unable to completely prevent the early cardiovascular changes attributed to medetomidine after intramuscular co-administration. This is probably because vatinoxan accelerates the rate of absorption and distribution of other simultaneously administered drugs, when they are injected intramuscularly (Salla et al. 2014b, Restitutti et al. 2017, Kallio-Kujala et al. 2018). Vatinoxan is believed to produce this effect by reducing vasoconstriction both systematically and locally at the injection site. However, vatinoxan itself is absorbed more slowly than medetomidine (Salla et al. 2014b, Restitutti et al. 2017, Kallio-Kujala et al. 2018). On the other hand, when vatinoxan was administered intravenously prior to or simultaneously with dexmedetomidine, or intramuscularly as separate injections prior to medetomidine administration, the early agonist-induced cardiovascular depression was absent or substantially reduced (Pagel et al. 1998, Enouri et al. 2008, Honkavaara et al. 2011, Rolfe et al. 2012). Hence, when medetomidine and vatinoxan are given together, the initial medetomidine-related haemodynamic changes may be avoided by intravenous administration of the drug combination. Thus, with intramuscular co-injection early medetomidine evoked cardiovascular changes are likely to occur, albeit attenuated by vatinoxan. Whilst problems related to drug absorption can be avoided with intravenous administration, it is often clinically a less practical administration route. Hence, further drug formulation development improving the rate of absorption of vatinoxan after intramuscular co-administration with medetomidine would likely be of benefit.

Vatinoxan reduced the peripheral vascular effects of medetomidine, demonstrated by decreased systemic vascular resistance index and mean arterial pressure (I, II), and attenuation of bradycardia (I – III). However, the central sympatholytic effects of medetomidine were unaffected, resulting in decreased heart rate when compared to baseline and also a temporal reduction in blood pressure that was particularly evident after ketamine administration. Honkavaara et al. (2011) demonstrated that when vatinoxan (250 µg kg⁻¹) was administered alone intravenously, heart rate increased and mean arterial blood pressure remained stable. Whereas, when vatinoxan was co-administered with dexmedetomidine (10 µg kg⁻¹), both heart rate and mean arterial pressure decreased significantly from baseline (Honkavaara et al. 2011). In study II mild hypotension (mean arterial pressures of 57–59 mmHg) was observed in two of the eight dogs with the treatment including vatinoxan 40 and 55 minutes after ketamine administration. This hypotensive effect was probably due to medetomidine’s central sympatholytic effect and vasodilative action of both vatinoxan and ketamine, all contributing to the outcome. A similar vasodepressor effect was observed during medetomidine-isoflurane anaesthesia in dogs: although the average mean arterial pressure remained above 60 mmHg, the decrease was significantly larger in the presence of vatinoxan (Salla et al. 2014a, Salla et al. 2017). Generally, the vasodilatory effect of commonly used
general anaesthetics might be masked by medetomidine’s vasoconstrictive effect that prevents hypotension. But since co-administration of vatinoxan potentiates the vasodilation, hypotension is more likely to occur, especially when medetomidine’s central sympatholytic effects dominate. Blood pressure is the product of cardiac output and systemic vascular resistance and usually flow-maintained pressure is preferred over resistance-maintained pressure to ensure tissue perfusion. In study II, during the hypotensive period, systemic vascular resistance was significantly lower and cardiac index significantly higher with treatment including vatinoxan. Whereas, without vatinoxan mean arterial pressure was significantly higher due to increased systemic vascular resistance while cardiac index was lower. These results suggest that following the improvement of cardiac index by vatinoxan, tissue perfusion was better maintained even during the mild hypotension (II). However, systemic hypotension is seldom a preferable event, so the vatinoxan-to-medetomidine dose ratio may need to be adjusted if medetomidine-vatinoxan combination is used as a premedication prior to general anaesthesia. Furthermore, whether or not mild hypotension coupled with a high cardiac index leads to deficits in oxygen delivery to e.g. the renal cortex or the central nervous system in dogs, would certainly warrant further investigations. Similarly, the use of antimuscarinic and other drugs commonly used to treat hypotension should be investigated in the presence of vatinoxan.

The difference between the two α₂-adrenoceptor antagonists vatinoxan and atipamezole used in the present studies, is that atipamezole antagonizes both central and peripheral α₂-adrenoceptors, whereas the effects of vatinoxan are limited mostly to the periphery (Clineschmidt et al. 1988). Previously, atipamezole has been used to reverse dexmedetomidine-induced sedation in the presence of vatinoxan, but without a comprehensive investigation of the cardiovascular effects (Honkavaara et al. 2008). When atipamezole was administered 30 minutes after medetomidine with the recommended 5:1 dose ratio, a transient decrease in mean arterial pressure was detected (I). A similar effect has been previously observed by others (Vainio 1990, Flacke et al. 1993, Hayashi et al. 1994a, Pyndop et al. 1996). Vainio (1990) proposed that it resulted from antagonism of the peripheral α₂-adrenoceptors and hence, reversal of vasoconstriction. In study I the momentary decrease in mean arterial pressure was of a similar magnitude both with and without vatinoxan. Hence, vatinoxan did not seem to potentiate or add to the vasodilatory effect of atipamezole. Systemic vascular resistance index, mean arterial pressure and heart rate returned to baseline within 30 to 45 minutes after atipamezole administration, demonstrating a beneficial cardiovascular interaction between vatinoxan and atipamezole (I).

When atipamezole was administered 60 minutes after anaesthetic induction with ketamine, equivalent to 80 minutes from premedication including vatinoxan, systemic vascular resistance index decreased slightly and blood pressure, heart rate and cardiac index increased (II). Hence, the observation that cardiovascular depression was reversed by atipamezole, indicates that medetomidine’s central cardiovascular depressor effects probably contributed to the decline in heart rate and cardiac index following ketamine’s initial effects. Mild hypotension detected 40 and 55 minutes after ketamine administration in the presence of vatinoxan was probably also attributable in part to medetomidine’s central sympatholytic effect. Similarly, the mild hypotensive period might further be explained by vatinoxan’s inability to antagonize the medetomidine-induced centrally mediated inhibition of catecholamine release thereby unveiling the vasodilatory action of ketamine. As atipamezole was able to reverse this effect, further studies measuring plasma catecholamine concentrations are needed to explore this theory.
6.2 Pulmonary effects of medetomidine, butorphanol and vatinoxan

Respiratory rate decreased after all treatments (I – III), probably due to medetomidine-induced depression of the respiratory centers in the central nervous system (Lerche and Muir 2006). Changes in arterial blood gas partial pressures were unaffected by vatinoxan as described previously by Honkavaara et al. (2011). However, when butorphanol was part of the premedication, a decrease in the partial pressure of oxygen and an increase in the partial pressure of carbon dioxide occurred (II). These changes were significantly larger 10 minutes after treatment administration with the addition of vatinoxan. This difference in the blood gas partial pressures may be attributed to higher initial butorphanol plasma concentrations, owing to improved intramuscular absorption in the presence of vatinoxan (II, III). Hence, the changes in arterial blood gas partial pressures were possibly the result of butorphanol’s influence on ventilation. Ko et al. (2000) reported that respiratory rate and arterial partial pressure of oxygen decreased, and arterial partial pressure of carbon dioxide increased in the presence of butorphanol in dogs sedated with medetomidine. These results are consistent with those reported by Salla et al. (2014b) where it was suggested that butorphanol promoted medetomidine-evoked hypoventilation. This effect was further exacerbated by the simultaneous administration of vatinoxan when the treatments were administered intramuscularly but was also noted when given intravenously (Salla et al. 2014b).

6.3 Influence of ketamine anaesthesia induction on medetomidine’s cardiopulmonary effects with and without vatinoxan

After the administration of intravenous ketamine (4 mg kg⁻¹) in study II, systemic vascular resistance index and mean arterial pressure decreased and heart rate and cardiac index increased with and without vatinoxan. Hence, haemodynamic function improved transiently after ketamine administration with both treatments. Increases in heart rate and cardiac index may be attributed to the central and peripheral sympathomimetic actions of ketamine, mediated by inhibition of norepinephrine uptake in adrenergic nerve endings (Salt et al. 1979). However, the decrease in systemic vascular resistance and mean arterial pressure, and the transient nature of the cardiostimulatory effects of ketamine may be explained by catecholamine depletion (Pagel et al. 1992), secondary to medetomidine’s centrally mediated sympatholytic effects. Hence, suppression of central and peripheral adrenergic neuronal transmission might unmask the direct vasodilatory and myocardial depressant actions of ketamine, independent of its sympathomimetic effects (Pagel et al. 1992).

Since vatinoxan does not reverse the initial hypertensive effect of medetomidine when administered intramuscularly, the simultaneous administration of ketamine might result in diverging cardiovascular outcome compared to study II. When intramuscular dexmedetomidine (15 μg kg⁻¹), buprenorphine (40 μg kg⁻¹) and ketamine 3 mg kg⁻¹ were given together, mean arterial pressures over 160 mmHg with dramatic decreases in cardiac output and arterial partial pressures of oxygen were observed (Ko et al. 2013). Given the cardiopulmonary outcome, the addition of ketamine to this combination was not considered to be beneficial (Ko et al. 2013). Adam et al. (2018) reported that concurrent administration of vatinoxan with medetomidine and ketamine in sheep alleviated
the adverse cardiopulmonary effects of the combination. A similar study with vatinoxan has not been conducted in dogs to the author’s knowledge. The timing of ketamine in relation to that of the \(\alpha_2\)-adrenoceptor agonist appears to influence its cardiovascular effects. Haskins et al. (1986) suggested that simultaneous administration of ketamine with \(\alpha_2\)-adrenoceptor agonists should be avoided. Thereby the simultaneous peak hypertensive effect of the two drugs, which appears to trigger the disadvantageous cardiovascular interaction, could be circumvented (Haskins et al. 1986). Hence, administering ketamine after the hypertensive effect of medetomidine has subsided appears to result in a better cardiovascular outcome. Since the cardiovascular effects were favourable after ketamine administration with both treatments in study II, medetomidine’s peak vasoconstrictive effects had probably already passed. With the treatment including vatinoxan, vasoconstrictive had been alleviated when ketamine was administered 20 minutes after the treatment (II).

Apnoea was detected after ketamine administration in six of the eight dogs with both treatments (II). This finding concurred with the study by Ko et al. (2001a) in which apnoea was observed in half of the dogs, following intravenous ketamine administration (4 mg kg\(^{-1}\)) after an intramuscular injection of medetomidine (10 µg kg\(^{-1}\)). Administration of vatinoxan did not affect the incidence or duration of respiratory depression after ketamine (II). Due to the apnoeic effect of intravenous ketamine, preoxygenation should be used along with means to support ventilation following intubation. However, the dose of ketamine chosen for study II was likely higher than required to achieve endotracheal intubation, which probably also contributed to the degree of respiratory suppression and may also have added to the hypotension discussed above.

6.4 Reversal of medetomidine’s undesirable cardiopulmonary effects with atipamezole and interactions of the two \(\alpha_2\)-adrenoceptor antagonists

After atipamezole administration in study I, systemic vascular resistance index and mean arterial pressure decreased and heart rate and cardiac index increased with both treatments. However, atipamezole failed to reverse medetomidine-induced changes in systemic vascular resistance index, heart rate or cardiac index without vatinoxan. In fact, heart rate and cardiac index declined again 30 minutes after atipamezole administration, with increases in systemic vascular resistance index and mean arterial pressure when vatinoxan was not included in the treatment. Hence, atipamezole failed to reverse any of the adverse medetomidine induced cardiovascular effects using the recommended 5-fold dose of medetomidine. A comparable finding was reported by Zatroch et al. (2019) when dexmedetomidine (5 µg kg\(^{-1}\)) was administered intravenously and followed by intramuscular administration of atipamezole (25 or 50 µg kg\(^{-1}\)) 5 minutes later in cats anaesthetized with isoflurane. Both doses of atipamezole were ineffective in increasing pulse rate or cardiac output, and resulted in short-lasting but severe arterial hypotension (Zatroch et al. 2019).

The timing of atipamezole administration following the \(\alpha_2\)-adrenoceptor agonist, as well as the administered dose ratio of atipamezole: \(\alpha_2\)-adrenoceptor agonist, seem to influence the net cardiovascular effect. Based on the results of studies I and II and in review of the literature, it is suggested that because atipamezole is a competitive \(\alpha_2\)-adrenoceptor antagonist
(Virtanen 1989), its dose needs to be adjusted according to the dexmedetomidine plasma concentration at the time of administration. Flacke et al. (1993) administered dexmedetomidine intravenously at increasing doses to enflurane-anaesthetized dogs. This was followed by an intravenous bolus of atipamezole at a 125:1 dose ratio (equivalent to 62.5:1 atipamezole:medetomidine). Atipamezole reversed all the systemic and coronary haemodynamic effects as well as the decrease in circulating catecholamine concentrations attributed to dexmedetomidine (Flacke et al. 1993). However, transient hypotension was detected following atipamezole administration and sometimes the cardiovascular response was exaggerated (Flacke et al. 1993). When atipamezole was administered intramuscularly 40 minutes after intramuscular medetomidine-midazolam combination at a 4:1 dose ratio to non-anaesthetized dogs, systemic vascular resistance decreased to baseline and mean arterial pressure decreased slightly below baseline levels (Hayashi et al. 1994a). Cardiac index and heart rate increased rapidly and remained above pre-atipamezole values (Hayashi et al. 1994a). Similar cardiovascular changes were also observed by Pypendop et al. (1996) after the intramuscular administration of atipamezole at a 2.5:1 dose ratio 60 minutes after the administration of medetomidine alone or combined with midazolam and butorphanol or buprenorphine. It was reported that systemic vascular resistance returned to baseline, mean arterial pressure decreased significantly, cardiac index increased but remained below baseline and heart rate increased but remained lower than baseline (Pypendop et al. 1996). Vainio and Vähä-Vahe (1990) observed that when atipamezole was administered intramuscularly 20 minutes after medetomidine, a 6:1 to 10:1 dose ratio was needed to maintain the heart rate permanently above pre-atipamezole levels. It was concluded in all of the aforementioned studies that atipamezole reversed the cardiovascular changes attributed to dexmedetomidine or medetomidine. However, the currently recommended 5:1 dose ratio of atipamezole:medetomidine in dogs, irrespective of the timing of atipamezole administration, might not be optimal for reversal of medetomidine or dexmedetomidine-induced cardiovascular changes. This was demonstrated by the inadequate effects of atipamezole when given 30 minutes after medetomidine in study I. Hence, further studies are still needed to determine the ideal atipamezole dose needed to reliably reverse the effects induced by α₂-adrenoceptor agonists, taking into account the time of atipamezole administration relative to dexmedetomidine plasma concentration. However, based on the cardiovascular results of study I, it appears that atipamezole is not very effective in reversing medetomidine’s peripheral effects. That is possibly due to its pharmacokinetic properties favoring central distribution and shorter duration of action than dexmedetomidine. In any case, vatinoxan appears to promote a far better reversal of the cardiovascular effects of medetomidine when atipamezole is administered.

Respiratory rates remained significantly decreased after atipamezole administration with both treatments (I). Similar results were reported by Hayashi et al. (1994a), who stated that atipamezole’s effects on respiratory function were minor following medetomidine and midazolam administration. However, other studies have demonstrated atipamezole’s ability to reverse the
respiratory depressant effects of medetomidine (Vainio 1990, Pypendop et al. 1996). The discrepancy might be explained by lack of physical activity of the dogs in study I, or indicate that atipamezole was not able to reverse the central respiratory depressant effects of medetomidine. The arterial partial pressures of oxygen and carbon dioxide, however, stayed within or close to normal reference ranges for dogs (Haskins et al. 2005) with both treatments throughout the study I.

6.5 Quality and duration of sedation, hypnosis and recovery

Composite sedation scores recorded in studies I and II, and visual analogue sedation scores measured in study III indicated that dogs became markedly sedated after administration of the treatments with and without vatinoxan. The results align with previous studies showing that vatinoxan does not substantially impair the quality of initial sedation (Honkavaara et al. 2008, Restitutti et al. 2011, 2017, Rolfe et al. 2012), attributed to limited passage of the drug to the central nervous system (Honkavaara et al. 2019). No differences in sedation between the treatments were noticed in studies I and II conducted in a laboratory environment. However, in the clinical trial, the sedative effect appeared sooner, and sedation was deeper during the first 30 minutes with the treatment that included vatinoxan (III). A similar finding that vatinoxan hastened the sedative effect of medetomidine when administered as a single intramuscular injection was observed by Restitutti et al. (2017). The depth of sedation probably increased with vatinoxan because of higher early plasma concentrations of other simultaneously administered drugs as demonstrated in study III. In study III, higher concentrations of medetomidine and butorphanol were measured in the presence of vatinoxan approximately 10 minutes after treatment administration. This is considered to result from accelerated absorption attributed to reversal of medetomidine’s local actions on the vasculature, leading to a faster peak sedative effect (Restitutti et al. 2017, Kallio-Kujala et al. 2018).

In the clinical trial, vatinoxan increased the need for additional sedation after a mean duration of 52 minutes following the initial treatment administration (III). The time-sedation curve showed that the dogs became alert sooner, and atipamezole was needed less often for the reversal when compared to the treatment without vatinoxan. Hence, the results of the clinical trial support the findings by Bennett et al. (2016) and Restitutti et al. (2017) that in the presence of vatinoxan, the duration of medetomidine-induced sedation was briefer. A consequence best explained by increased clearance of drugs, due to improved haemodynamic function. Overall, the recoveries from sedation were uneventful in studies I and III. In study III, a larger percentage of dogs were lethargic according to their owners in the absence of vatinoxan during the evening after the study.

Vatinoxan did not affect the quality of anaesthetic induction or conditions for endotracheal intubation when ketamine (4 mg kg\(^{-1}\)) was administered intravenously 20 minutes after treatment (II). However, the duration of anesthesia was shortened by vatinoxan. Similar to the above, this is probably attributed to its antagonism of the cardiovascular depressant effects of medetomidine (Honkavaara et al. 2012). Early and late recovery scores following ketamine anaesthesia were not affected by vatinoxan (II). However, some dogs with both treatments showed signs of
gastrointestinal side effects (nausea, defaecation and mucoid faeces) after atipamezole administration, which decreased the overall quality of recovery. Atipamezole induces defaecation in dogs by abolishing medetomidine’s inhibitory effect on colonic motility (Maugeri et al. 1994). Hence, vatinoxan may restore gastrointestinal motility by antagonizing the peripheral \( \alpha_2 \)-adrenoceptors and thus increasing the colonic smooth muscle tone. Administration of an \( \alpha_2 \)-adrenoceptor antagonist may also prevent the inhibitory influences of the sympathetic nervous system on colonic motility, inducing migrating contractions and leading to defaecation (Maugeri et al. 1994). Hector et al. (2017) reported that vomiting and defaecation were observed in half of the dogs following recovery from isoflurane anaesthesia during which vatinoxan had been administered alone as an intravenous infusion. Since ketamine can also cause salivation and defaecation in dogs (Haskins et al. 1985, Jacobson and Hartsfield 1993), the observed gastrointestinal side effects were probably due to interactions between the drugs. The gastrointestinal side effects were then further triggered by atipamezole administered at a 5-fold medetomidine dose, 80 minutes after treatment administration (Ⅱ). Gastrointestinal side-effects were not detected when atipamezole was used at an identical dose to reverse medetomidine’s effects 30 minutes after treatment administration with or without vatinoxan (Ⅰ).

Due to the gastrointestinal side effects observed in study Ⅱ, atipamezole was administered at a lower dose in the clinical trial (Ⅲ). Hence, atipamezole was administered at a 1.25-fold medetomidine ratio to reverse the sedation after completion of the procedure, followed by repeated administration if warranted (Ⅲ). No gastrointestinal side effects were observed during the recoveries, but the results of the owner questionnaire showed that all dogs given vatinoxan, atipamezole or both of the \( \alpha_2 \)-adrenoceptor antagonists had loose faeces after the procedure. However, since all of the dogs given vatinoxan (n = 27) as part of their treatment, and 26 out of the 28 dogs that were not given vatinoxan needed sedation reversal with atipamezole, it is impossible to assess if or how the changes in faecal consistency were actually due to \( \alpha_2 \)-adrenoceptor antagonist administration. Furthermore, the actions of medetomidine and/or butorphanol and individual sensitivity to gastrointestinal disturbances may have influenced the results.

6.6 Drug concentrations in plasma and clinical outcome
Plasma concentrations of dexmedetomidine and butorphanol increased more rapidly in the presence of vatinoxan when the drugs were given as a single intramuscular injection (Ⅲ). The clinically observed benefit was hastened onset of the greater sedative effect. However, in the laboratory study, the aforementioned phenomenon was also associated with some respiratory depression. This was evident as an increase in the partial pressure of carbon dioxide and decrease in the arterial partial pressure of oxygen, when butorphanol was included in the premedication and dogs were breathing room air (Ⅱ). Enhancement of the absorption of co-administered drugs with vatinoxan has been evidenced by a significantly shorter time to maximum drug concentration in plasma, associated with the timing of the peak sedative effect (Restitutti et al. 2017, Kallio-Kujala et al. 2018). Restitutti et al. (2017) proposed that this was due to prevention of local vasoconstriction.
induced by medetomidine. Nevertheless, supplemental oxygen may be necessary to prevent hypoventilation-induced hypoxemia in dogs sedated with this combination.

The plasma concentrations of ketamine were lower in the presence of vatinoxan 10 minutes after intravenous administration (II). And overall, a faster decline in the concentrations of co-administered drugs in the presence of vatinoxan was observed. This was also true when the plasma concentrations of dexmedetomidine and butorphanol were measured after the absorption phase had passed (I, II). Clinically this manifested as shorter duration of medetomidine-induced sedation and ketamine-evoked hypnosis compared to treatments without vatinoxan (III, II).

Bennett et al. (2016) reported that co-administration of vatinoxan had significant effects on the disposition of dexmedetomidine (when the racemate was administered), demonstrated by an increased volume of distribution and clearance, as well as a reduction in the elimination half-life and area under the time-concentration curve. Honkavaara et al. (2012) proposed that increased clearance is probably attributed to preserved liver blood flow by vatinoxan. The results of studies I and II showed that vatinoxan reduced the exposure to other drugs. That was demonstrated by significantly smaller area under the time-concentration curve of ketamine, when compared to the treatment without vatinoxan.

After atipamezole was administered 30 minutes after medetomidine-vatinoxan, dexmedetomidine concentrations were significantly lower throughout the observation period in the presence of vatinoxan (I). With vatinoxan plasma dexmedetomidine concentrations peaked before atipamezole administration, whereas in the absence of vatinoxan the peak dexmedetomidine plasma concentration was not observed until 5 minutes after atipamezole administration. The higher dexmedetomidine plasma concentrations after atipamezole administration were observed clinically as somnolence. Resedation after an initial period of arousal, demonstrated by a recurring increase in composite sedation scores 30 minutes after atipamezole administration, was observed with the medetomidine-only treatment. In the presence of vatinoxan, resedation was not observed as demonstrated by the steady decline in composite sedation scores after atipamezole administration. Hence, the recovery was more complete with the medetomidine-vatinoxan treatment. In the absence of vatinoxan, mean plasma concentrations of dexmedetomidine throughout the 210 minute observation period remained above 0.5 ng mL⁻¹, which has been associated with sedation in dogs by Van Oostrom et al. (2011). With the treatment including vatinoxan, the plasma dexmedetomidine concentrations decreased below 0.5 ng mL⁻¹ within 90 minutes following atipamezole administration. In clinical settings, incomplete recoveries from short procedural sedations are a cause for concern. By increasing the clearance of co-administered sedatives, vatinoxan may ensure more complete recoveries.

### 6.7 Methodological considerations

In studies I and II the lithium dilution method was used to measure cardiac output. When lithium dilution and thermodilution methods were compared during hypodynamic, normodynamic and hyperdynamic cardiovascular states in dogs, LiDCO agreed well with the transpulmonary and
traditional thermodilution methods (Morgaz et al. 2014). Background lithium concentration may result in overestimation of cardiac output by LiDCO in case the limit of 0.2 mmol L\(^{-1}\) plasma lithium concentration is exceeded, according to the manufacturer. However, Mason et al. (2002) showed that 34 injections of lithium chloride at a routine dose during 3-7 hours were needed to reach lithium concentrations equal or above the limit. Maximum of eight injections with a standard lithium chloride dose were given over 1.5 hours in studies I and II. Hence, plasma lithium chloride concentration was unlikely to exceed the limit reported to alter the cardiac output measurements. Ambrisko et al. (2013) published the results of an in vitro study showing that the \(\alpha_2\)-adrenoceptor agonist xylazine and ketamine produced a positive bias of over 10% in the LiDCO voltage sensor. While the effects of ketamine and xylazine on the LiDCO sensor were additive. Dexmedetomidine, butorphanol and atipamezole were considered unlikely to interact with the LiDCO sensor (Ambrisko et al. 2013). The plasma concentrations of ketamine indicate that a positive bias of 15 % in study II until 40 minutes after ketamine administration was theoretically possible. Since the plasma concentration of ketamine was higher in the absence of vatinoxan, the positive bias was likely to be smaller in the presence of vatinoxan. And thus, unlikely to falsely increase the difference in cardiac output between treatments. However, the in vitro findings of Ambrisko et al. (2013) have not been verified in vivo in dogs.

There were some further protocol and methodology issues related to the present studies. In study I dogs were manually restrained gently on an examination table because of continuous cardiovascular measurements until 90 minutes after atipamezole administration, which obviously prevented physical activity during the later observational period. In turn, however, this enabled us to better detect which cardiovascular changes resulted from drug administration, since in a clinical scenario the surroundings and patient activity likely affect the cardiovascular outcome. One of the limitations in study II was that there was no group that did not receive ketamine to compare the results with. However, Salla et al. (2014b) had previously performed a study investigating the cardiopulmonary effects of medetomidine and butorphanol with and without vatinoxan. In that study the drug dosages and routes of administration were identical to the present study. Therefore, we were able to draw some comparisons with that study although, it did not compensate for this shortcoming. Another limitation of study II was that we administered a predetermined dose of ketamine to all dogs rather than administering ketamine to effect (i.e. to the point where endotracheal intubation was possible). Thus, we were not able to compare the ketamine doses needed for successful intubation between treatments. This decision was taken because the primary focus of the study was to detect differences in cardiovascular effects between treatments. The dose of ketamine was chosen to ensure detection of any haemodynamic impact by vatinoxan. Salla et al. (2017) had already observed that the average ketamine dose needed for endotracheal intubation in medetomidine pre-treated dogs with and without vatinoxan was 1 mg kg\(^{-1}\) when administered simultaneously with midazolam 0.2 mg kg\(^{-1}\). Hence, there was no need to repeat the same investigation in the present study.
The environment of clinical teaching hospital and the fact that client-owned dogs were studied, place more restrictions on study design and protocol when compared with laboratory settings. A major limitation of study III was the lack of blood pressure data. We did not consider the data we obtained to be reliable for following reasons: blood pressure was measured non-invasively and the measurements had to be performed without excessive interference with the radiological examinations. This led to variations in data collection time points and because the position of the dog varied, we could not use the same sites for the blood pressure measurements in every dog. Furthermore, medetomidine causes cardiovascular changes influencing both cardiac rhythm and the blood flow in the vessels, which makes it more difficult to obtain reliable data with oscillometric devices. Hence, there was a large variation in the blood pressure readings. Additionally, as we did not instrument the dogs before treatment administration in study III, the blood sample collection times for plasma drug concentration analysis varied, depending on the ease of intravenous catheter placement. Still, blood sampling time points remained within acceptable limits compared to the predetermined blood sampling time point of 10 minutes after treatment administration. Most importantly, the actual sampling times were not different between treatments.

One of the limitations of studies I – III was that even though blood samples were collected to measure drug concentrations in plasma, the studies were not designed as pharmacokinetic investigations. Limited number of blood samples for drug analysis were collected in short time period after drug administration. Therefore, only few pharmacokinetic parameters could be calculated. This was a deliberate decision, because the emphasis of the studies was on haemodynamics. In studies I and II, the measurement of cardiac output was preferred, since only a restricted volume of blood could be drawn from the dogs. Sole purpose of the drug concentration analysis was to detect the influence of vatinoxan on plasma concentrations of co-administered drugs, and to enable reflection between clinical observations and plasma drug concentrations.

### 6.8 Clinical implications and relevance
The addition of vatinoxan to sedation or preanaesthetic protocols influences the cardiovascular outcome as well as the intensity and duration of the effects of co-administered drugs. Hence, it is important that veterinarians are aware of all vatinoxan-evoked changes.

### 6.8.1 Medetomidine-vatinoxan as sedative combination in dogs
In the clinical trial it was demonstrated that the combination of medetomidine 0.5 mg m⁻² (equivalent of approximately 20 µg kg⁻¹ medetomidine for a 10–20 kg dog) and vatinoxan 10 mg m⁻² with butorphanol 0.1 mg kg⁻¹ provided reliable sedation for diagnostic imaging in client-owned dogs. (III). The time when the dog laid its head down was used as an indicator of the onset of sedation. It was approximately 4 ± 1 minutes in the presence of vatinoxan and 5 ± 1 minutes without vatinoxan. In contrast, Kuo and Keegan (2004) observed that the interval from intravenous administration of medetomidine 20 µg kg⁻¹ and butorphanol 0.2 mg kg⁻¹ until lateral recumbency was 1.4 ± 0.4 minutes. When the onset time after intravenous and intramuscular administration of medetomidine
and butorphanol, as well as the feasibility and the cardiopulmonary outcome are taken into account, intramuscular administration may be clinically preferable. The results of the clinical trial showed that vatinoxan hastened the onset of the sedative effect of medetomidine and butorphanol, which makes the intramuscular administration route even more favorable. That said, if intravenous co-administration is an option, vatinoxan may be able to fully prevent the early haemodynamic depression attributed to medetomidine.

Deeper overall sedation was observed in the presence of vatinoxan during the first 30 minutes following treatment administration, as demonstrated by a significantly larger area under the time-sedation curve for sedation in the presence of vatinoxan (III). However, if the procedure lasted longer than 50 minutes, additional sedation was required more often with than without vatinoxan. Kuo and Keegan (2004) reported that the mean duration of lateral recumbency after intravenous medetomidine 20 µg kg⁻¹ and butorphanol 0.2 mg kg⁻¹ administration was approximately 140 ± 20 minutes. Hence, the duration of the sedative effect of the medetomidine-butorphanol combination appeared substantially reduced by the co-administration of vatinoxan. Therefore, the medetomidine-vatinoxan combination seems best suited for shorter clinical procedures. On the other hand, dogs that were given vatinoxan were more alert after completion of the diagnostic imaging procedure and hence, atipamezole was required to reverse sedation less often (III). Sedation needed to be reversed in 90% of the dogs that were not given vatinoxan and in 44% of the dogs that had were given vatinoxan. In study I it was observed that atipamezole reversed medetomidine-induced sedation more completely with vatinoxan. Thus, co-administration of vatinoxan might improve the clinical usability of medetomidine for brief procedural sedation, when fast recovery from sedation is required.

The apparent resedation and sustained haemodynamic compromize after administration of atipamezole observed in study I were marked in the absence of vatinoxan. Hence, regardless of atipamezole administration, the dogs might experience medetomidine’s sedative and cardiovascular depressive effects after discharge from the clinic, since in clinical practice dogs are commonly discharged soon after or during the initial arousal. This might render patients vulnerable to uncontrolled complications without veterinary supervision. In study I atipamezole was able to reverse the sedative and cardiovascular effects more efficiently when medetomidine and vatinoxan were administered in combination, potentially improving the clinical safety of short medetomidine-based procedural sedations.

In the clinical trial vatinoxan alleviated the medetomidine-induced bradycardia (III). Mean heart rate was approximately 60 beats minute⁻¹ with vatinoxan, compared to 40 beats minute⁻¹ without it. Although cardiac output was not measured in the clinical trial (III), the association between heart rate and cardiac output in medetomidine-sedated dogs was obvious in the present laboratory studies (I, II) and in various other experimental studies (Honkavaara et al. 2011, Rolfe et al. 2012, Salla et al. 2014b, Restitutti et al. 2017). Hence, the vatinoxan-increased heart rate is likely associated with improved cardiovascular function in medetomidine-sedated dogs in clinical practice.
6.8.2 Medetomidine-vatinoxan-butorphanol combination as premedication prior to anaesthesia with ketamine

Overall, vatinoxan improved cardiovascular function before and after ketamine in dogs premedicated with medetomidine and butorphanol, as shown by a higher heart rate and cardiac index. That said, vatinoxan was associated with greater reductions in systemic vascular resistance index and sporadic hypotension, the latter being observed rather late after the administration of ketamine. Thus, it is likely that the peripheral vasodilation by vatinoxan will add to any centrally mediated cardiovascular depression caused by commonly used anaesthetics. Clinically this may manifest as an increased incidence of hypotension during anaesthesia in dogs premedicated with medetomidine-vatinoxan combination, especially if drugs with vasodilatory, sympatholytic or negative inotropic or chronotropic effects are administered simultaneously. Salla et al. (2014a) reported that during isoflurane anaesthesia in dogs, the cardiovascular effects of medetomidine-vatinoxan premedication were very similar to those of acepromazine-butorphanol premedication. Hence, it is important for clinicians to recognize that the cardiovascular outcome during anaesthesia after medetomidine-vatinoxan premedication might be distinctly different from that of medetomidine alone. Anaesthesia was not maintained by inhalant anaesthetic in the present study. However, it is worth noting that Hector et al. (2017) reported that vatinoxan administration as a constant rate infusion (180 µg kg⁻¹ h⁻¹) in dogs during sevoflurane anaesthesia increased the minimum alveolar concentration of the inhalant by approximately 20% in dogs. Further studies are needed to determine the clinical relevance of this observation by Hector et al. (2017).

Vatinoxan shortened the anaesthetic effect of medetomidine-butorphanol-ketamine combination (Ⅱ). Hence, vatinoxan might also influence the effects of other anaesthetics divergent to medetomidine alone. Restitutti et al. (2013) observed that dexmedetomidine-induced decreases in organ blood flow, including the liver and kidneys, were attenuated by vatinoxan. For xenobiotics with high hepatic extraction ratios, plasma clearance rate is dominated by drug delivery regulated by liver blood flow (Wilkinson 1987). Hence, drugs that decease hepatic blood flow impair hepatic drug clearance. For example, Bennett et al. (2017) reported that vatinoxan increased volume of distribution and clearance of alfaxalone in dogs given constant rate infusions of alfaxalone, medetomidine and vatinoxan. The clinical relevance of the aforementioned finding is that vatinoxan might reduce the anaesthetic-sparing effect of simultaneously administered drugs by decreasing their exposure. Hence, the dose of anaesthetic drugs may need to be adjusted in presence of vatinoxan. Co-administration of vatinoxan also decreases concentrations of dexmedetomidine in plasma after the absorption phase, as observed in studies I and Ⅱ. Therefore, the later sedative and antinociceptive effects of medetomidine are decreased and shortened (Bennett et al. 2016), which needs be taken into consideration in clinical decision-making. For instance, dogs may need additional pain relief sooner in the presence of vatinoxan. Also, the recoveries from general anaesthesia may be more restless if the sedative effect of medetomidine has already subsided.
7 CONCLUSIONS

1. Atipamezole failed to reverse medetomidine-induced cardiovascular changes and prevent resedation. Coadministration of vatinoxan with medetomidine helped to restore haemodynamic function and hastened the recovery from sedation after atipamezole administration. Atipamezole and vatinoxan interacted favorably, without cardiovascular adverse effects. (Ⅰ)

2. Vatinoxan and ketamine improved haemodynamic function in dogs following medetomidine-butorphanol premedication. Vatinoxan was associated with mild hypotension in a minority of the dogs. Gastrointestinal adverse effects such as nausea and defeacation after atipamezole administration decreased the quality of recovery in some dogs, both with and without vatinoxan. (Ⅱ)

3. Concurrent intramuscular administration of vatinoxan with medetomidine and butorphanol in client-owned dogs hastened the onset of peak sedation intensity, alleviated the bradycardia and provided reliable sedation for short term clinical procedures. Atipamezole was required less frequently to reverse sedation when vatinoxan was co-administered with medetomidine. (Ⅲ)

4. Vatinoxan increased the early stage plasma concentrations of intramuscularly co-administered drugs (Ⅰ,Ⅱ). However, the plasma concentrations of simultaneously administered drugs also decreased sooner, probably due to improved haemodynamic function. The clinical outcome was a faster onset and shorter effect of the presently investigated centrally acting drugs (Ⅰ–Ⅲ).
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Cardiovascular and sedation reversal effects of intramuscular administration of atipamezole in dogs treated with medetomidine hydrochloride with or without the peripheral $\alpha_2$-adrenoceptor antagonist vatinoxan hydrochloride

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OBJECTIVE
To investigate the cardiovascular and sedation reversal effects of IM administration of atipamezole (AA) in dogs treated with medetomidine hydrochloride (MED) or MED and vatinoxan (MK-467).

ANIMALS
8 purpose-bred, 2-year-old Beagles.

PROCEDURES
A randomized, blinded, crossover study was performed in which each dog received 2 IM treatments at a $\geq$ 2-week interval as follows: injection of MED (20 µg/kg) or MED mixed with 400 µg of vatinoxan/kg (MEDVAT) 30 minutes before AA (100 µg/kg). Sedation score, heart rate, mean arterial and central venous blood pressures, and cardiac output were recorded before and at various time points (up to 90 minutes) after AA. Cardiac and systemic vascular resistance indices were calculated. Venous blood samples were collected at intervals until 210 minutes after AA for drug concentration analysis.

RESULTS
Heart rate following MED administration was lower, compared with findings after MEDVAT administration, prior to and at $\geq$ 10 minutes after AA. Mean arterial blood pressure was lower with MEDVAT than with MED at 5 minutes after AA, when its nadir was detected. Overall, cardiac index was higher and systemic vascular resistance index lower, indicating better cardiovascular function, in MEDVAT-atipamezole–treated dogs. Plasma dexmedetomidine concentrations were lower and recoveries from sedation were faster and more complete after MEDVAT treatment with AA than after MED treatment with AA.

CONCLUSIONS AND CLINICAL RELEVANCE
Atipamezole failed to restore heart rate and cardiac index in medetomidine-sedated dogs, and relapses into sedation were observed. Coadministration of vatinoxan with MED helped to maintain hemodynamic function and hastened the recovery from sedation after AA in dogs. (Am J Vet Res 2019;80:912–922)

Procedural sedation is often needed in small animal clinical practice to allow safe and effective animal handling without undue stress. Rapid and smooth postprocedural recovery from sedation is advanta-gous for the animal, its owner, and the veterinary staff. One of the main benefits of $\alpha_2$-adrenoceptor agonists, such as MED and its pharmacologically active enantiomer dexmedetomidine, is the reversibility of sedation by administration of the $\alpha_2$-adrenoceptor antagonist atipamezole.1,2 Disadvantages of $\alpha_2$-adrenoceptor agonists are their cardiovascular adverse effects, such as vasoconstriction, bradycardia, and decreased cardiac output,5,6 that limit their safe use especially in animals with systemic disease.

Atipamezole is a potent and specific antagonist of centrally and peripherally located $\alpha_2$-adrenoceptors.2 It reverses the sedative and some cardiovascular and respiratory effects induced by MED,5,7 thereby facilitating more rapid recovery from MED-induced sedation.4,8 However, transient decreases in blood pres-
sure soon after atipamezole administration, which are thought to be caused by atipamezole’s vasodilatory effect, have been reported.17-19

The peripheral α2-adrenoceptor antagonist vatinoxan (also known as MK-4677 and L-659066)8 alleviates the undesired cardiovascular depression associated with dexmedetomidine and MED11-13 without substantially altering the level of sedation.14-16 Vatinoxan crosses the blood-brain barrier poorly in mammals and has antagonistic action only at α2-adrenoceptors located in peripheral tissues,10 which explains the differences in clinical outcomes after administration of vatinoxan or atipamezole. Vatinoxan has distinct effects on the pharmacokinetics of MED and dexmedetomidine (eg, by increasing drug absorption after IM administration of either α2-adrenoceptor agonist and by promoting drug clearance),17-20 which result in more rapid onset and shorter duration of sedation.18,21 Concurrent administration of vatinoxan improves the tolerability and attenuates the adverse cardiovascular effects of MED and dexmedetomidine in dogs and cats.11,13,22 However, when sedation is reversed with atipamezole in animals treated with vatinoxan and MED or dexmedetomidine, vatinoxan might amplify the reduction in blood pressure attributed to atipamezole because both agents induce vasodilatation by blocking vascular α2-adrenoceptors.7,13

It has been reported that atipamezole reverses sedation in dogs sedated with dexmedetomidine and vatinoxan,14 but there are no reports on the possible cardiovascular consequences of concomitant administration of vatinoxan and atipamezole in dogs sedated with α2-adrenoceptor agonists, to our knowledge. The purpose of the study reported here was to investigate the cardiovascular and sedation reversal effects of IM administration of atipamezole in dogs treated with MED or MEDVAT. To this end, the influences of vatinoxan and atipamezole on cardiovascular functions, respiration, sedation-related factors, and plasma drug concentrations in dogs sedated with MED were evaluated. We hypothesized that treatment with atipamezole and vatinoxan would improve cardiovascular performance by decreasing SVRI, thereby increasing both heart rate and CI, without inducing clinically relevant hypotension. We also expected that vatinoxan administration would not have any significant effect on respiration. Atipamezole is known to increase the clearance and decrease the elimination half-life of MED.23 Consequently, we further hypothesized that by improving perfusion in peripheral tissues, vatinoxan would decrease the plasma exposure to MED and thus hasten recovery from MED-induced sedation in dogs.

Materials and Methods

Animals

Approval (EASVI/7187/04.10.03/2012) from the National Animal Experimental board to use 8 purpose-bred Beagles for the study was granted. Dogs were approximately 2 years old and had a mean ± SD body weight of 14.7 ± 1.5 kg. They were considered healthy on the basis of history and results of a comprehensive general examination that included a CBC and routine serum biochemical analyses. Dogs were housed in groups in a kennel with daily maintenance and activities. They were fed commercial food and had free access to water. Food was withheld 12 hours prior to experiments. After each experimental day was completed, the dog was assessed for recovery (heart rate and CSS) from sedation and fed and meloxicam (0.2 mg/kg) was administered SC for analgesia before transfer back to the kennel. After completion of the study, all dogs were placed with families in homes.

Study design

In this prospective, randomized,8 blinded, experimental crossover study, each dog received 2 IM treatments at an interval of ≥2 weeks. On each of the 2 experimental days, each dog was treated IM with MED hydrochloride6 (20 µg/kg) or MED (20 µg/kg) mixed with vatinoxan hydrochloridec (400 µg/kg) 30 minutes before administration of atipamezole hydrochloride4 (5 mg/mL). One dog was evaluated on each experimental day, and the overall duration of the study was 2 months.

Medetomidine-vatinoxan solution was prepared for each dog undergoing the MEDVAT treatment on a given day just before administration by injecting 1 mL of MEDb solution and 1 mL of physiologic saline (0.9% NaCl) solution into an ampule containing 20 mg of vatinoxan powder and mixing the ampule contents until the solution was clear. The final drug concentration in the solution was 500 µg of MED/mL and 10 mg of vatinoxan/mL; hence, the injection volume was 0.04 mL/kg. The MED-to-vatinoxan dose ratio of 1:20 was selected on the basis of previously published experimental data.18 Atipamezole was administered according to information in the product characteristics summary4 at the recommended dose of 5 times the preceding dose of MED.

Instrumentation

At the start of an experiment, each dog was placed on an examination table covered with an isolating foam mattress and heating pad. A cephalic vein was cannulated with a 22-gauge IV catheter. By use of a face mask, each dog was allowed to breathe 100% oxygen (5 L/min) before induction of anesthesia with propofol administered IV to effect (maximum dose, 8 mg/kg). The dog’s trachea was intubated, and anesthesia was maintained by inhalation of isoflurane in oxygen (end-tidal isoflurane concentration, 1.5%) via a circle breathing system. Each dog was mechanically ventilated to maintain end-tidal Paco2 between 35 and 45 mm Hg. Monitoring (noninvasive blood pressure assessment, ECG, and pulse oximetry) and IV infusion of acetated Ringer solution (5 mL/kg/h) were started. A 20-gauge arterial catheter6 was aseptically
introduced into a femoral artery under local anesthesia and secured in place with 2 sutures and surgical tape. The arterial catheter was used for direct arterial blood pressure and cardiac output measurements and collection of blood samples for arterial blood gas analysis. Local anesthetic (0.25 mL of 2% lidocaine solution) was injected SC to mark the place for a small skin incision over a jugular vein. A 17F double-lumen central venous catheter was inserted aseptically into the jugular vein and sutured in place; a light bandage was applied around the neck. To ensure the accurate location of the catheter's tip, the insertion site of the central venous catheter was premeasured and marked to indicate positioning of the tip of the catheter at the cranial border of the second rib's costochondral junction. Confirmation of a typical pressure waveform was performed after insertion. The central venous catheter was used for measurement of CVP and collection of venous blood samples for assessment of plasma drug concentrations. Isoflurane administration was discontinued after placement of both catheters. Full recovery from anesthesia (characterized by the dog's ability to walk and interact normally) was ensured by allowing the dog to recover for at least 60 minutes after extubation prior to obtaining baseline measurements.

Measurements

Prior to each experiment, blood pressure transducers were calibrated with a mercury manometer and zeroed to atmospheric pressure at the level of the manubrium. Each dog was positioned on the examination table in lateral recumbency with minimal gentle restraint and connected to the blood pressure transducers for continuous measurement of central venous pressure, MAP, DAP, and SAP. For ECG monitoring, adhesive ECG electrodes were placed on the forelimbs and left hind limb; the lead II tracing was monitored continuously. When the dog was lying calmly on the table, heart rate and blood pressure were allowed to stabilize before baseline measurements were obtained. After recording of all baseline values and collection of blood samples, MED or MEDVAT was injected into the right gluteal muscle; 30 minutes later, atipamezole was injected into the left gluteal muscle (designated as the 0-minute time point). Prior to any drug injection, negative pressure was applied to the syringe; if no blood was apparent, needle placement for extravascular drug administration was confirmed. For each dog, a heating pad and blankets were used to maintain normothermia.

At data collection time points when multiple variables were assessed, heart rate (auscultated heart beats counted during a 1-minute period), respiratory rate (thoracic movements counted during a 1-minute period), and blood pressures were always recorded first. These variables were assessed before (~30 [baseline] and ~10 minutes) and after (5, 10, 15, 20, 30, 45, 60, and 90 minutes) administration of atipamezole. Heart rate was also determined at the end of the study (210 minutes after atipamezole administration). Cardiac output was measured as described previously at baseline and ~10, 5, 15, 30, 45, 60, and 90 minutes by the lithium dilution method with a standard dose of lithium chloride (0.075 mmol) injected via the central venous catheter. Initial standard values of 10 g/dL for hemoglobin concentration and 140 mmol/L for sodium concentration were later corrected with actual values obtained from arterial blood samples. Just prior to measurements of cardiac output, arterial blood samples were collected into heparinized syringes and analyzed immediately for blood gases. Cardiac index, RPP, stroke volume index, and SVRI were calculated by use of standard equations.

For each dog, a venous blood sample (6 mL) for plasma drug concentration analyses was collected from the central venous catheter into EDTA tubes before (~10 minutes) and after (5, 10, 15, 20, 30, 60, 90, and 210 minutes) administration of atipamezole. Venous blood samples were centrifuged, and plasma was separated into tubes that were kept frozen at ≤−20°C until analyzed for dexmedetomidine, levomedetomidine, vatinoxan, and atipamezole concentrations.

Each dog was assigned a CSS (ranging from 0 [no sedation] to 20 [deep sedation]) by an investigator (HT) who was unaware of the drug treatment or any values of cardiovascular variables before (~30 and ~10 minutes) and after (5, 10, 15, 20, 30, 45, 60, and 90 minutes) atipamezole administration. Each dog was assigned a CSS by an unblinded investigator (KN) at 210 minutes after atipamezole administration to ensure full recovery from sedation. For each dog, a total CSS (Appendix) was calculated as the sum of scores for spontaneous posture (0 to 4), palpebral reflex (0 to 3), position of the eye (0 or 2), jaw and tongue relaxation (0 to 4), resistance to positioning in lateral recumbency (0 to 3), and general appearance including response to noise (0 to 4). The maximum achievable total score was 20.

Concentrations of dexmedetomidine, levomedetomidine, and vatinoxan in plasma samples were determined by liquid chromatography coupled with tandem mass spectrometry after solid-phase extraction. Racemic deuterium-labeled MED was used as internal standard for dexmedetomidine and levomedetomidine. An internal standard for vatinoxan was also used. Reversed-phase separation and a gradient solvent system (0.1% formic acid in water and acetonitrile) were used before quantitative detection of vatinoxan. Chiral separation of dexmedetomidine and levomedetomidine was achieved by use of 10mM ammonium acetate and acetonitrile as solvents. Quantitative detection of the analytes was performed in multireaction monitoring mode with a triple quadrupole mass spectrometer. For dexmedetomidine and levomedetomidine and for deuterium-labeled MED, the m/z of the respective precursor ions were 201.2 and 204.2, respectively. The m/z for monitored fragment ions used for quantitation
were 95.1 for dexmedetomidine and levome-detomidine and 98.05 for the internal standard. The linear concentration ranges for dexmedetomidine and levome-detomidine were 0.100 ng/mL to 10.0 ng/mL. The accuracy of the quality control samples (at 0.225, 1.0, and 8.0 ng/mL) ranged from 91.3% to 99.1% for dexmedetomidine and from 94.6% to 104.2% for levome-detomidine. The linear concentration range of the assay for vatinoxan was 25 ng/mL to 460 ng/mL. For vatinoxan, the interassay accuracy of the quality control samples (at 70, 250, 380, and 2500 ng/mL) ranged from 96.9% to 112%.

Concentrations of atipamezole in plasma samples were analyzed by liquid chromatography coupled with tandem mass spectrometry after precipitation of a 100-µL volume of each sample with 200 µL of acetonitrile containing 100 ng of propranolol/mL as the internal standard on a 96-well precipitation plate. After mixing, the samples were kept in a refrigerator for 20 minutes at 8°C, and then centrifuged at for 20 minutes. The supernatants were transferred to 96-well plates, and 50-µL aliquots were transferred to another 96-well plate and diluted 1:20 with 20% acetonitrile in PBS solution (pH, 7.4) prior to analysis. Both diluted and undiluted samples were analyzed. Reference standards and quality control samples were prepared in blank dog plasma. The selected reaction monitoring was m/z of 215 > 117 for atipamezole and m/z of 260 > 116 for internal standard propanolol. Quantitation was based on the peak area ratios of the analyte and the internal standard. The calibration range was 0.1 to 5,000 ng/mL, and all quality control samples (at 0.2, 2, 20, 200, and 2,000 ng/mL) were within 91% to 115% of the nominal concentration.

**Statistical analysis**

Sample size calculations were performed on the basis of data from our research group’s previous studies. Eight dogs were used to detect differences (peak effects) between treatments (with a paired 2-tailed test, α of 0.05, and power of 80%) as follows: difference in heart rate of 11 beats/min (50

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**Figure 1**—Mean ± SD heart rate (A), CI (B), MAP (C), and SVRI (D) in 8 dogs that received an IM injection of MED (20 µg/kg [squares]) or MED mixed with 400 µg of vatinoxan/kg (MEDVAT [circles]) 30 minutes before IM administration of atipamezole (100 µg/kg [indicated at 0 minutes by the triangle]) at a ≥ 2-week interval in a randomized crossover study. Error bars indicate the 95% confidence intervals of the means. Variables of interest were measured at various time points before (at –30 minutes [baseline] and –10 minutes) and at various time points after atipamezole administration. Values of CO and body surface area were used to calculate CI, and values of MAP, CVP, and CI were used to calculate SVRI. *At a given time point, there is a significant (P < 0.05) difference between treatments. †At a given time point for a given treatment, there is a significant (P < 0.05) difference from baseline.
The estimates of treatment effects were calculated over time and by time point from the fitted models with contrasts. Furthermore, within-group changes were calculated for each time point from the same models. For the group differences and within-group changes, 95% confidence intervals and P values were calculated. Differences in AUCs of drug concentrations and CSSs between the treatments were compared with paired 2-tailed *t* tests. Significance was set at a value of *P* < 0.05. Computer software was used for all statistical analyses.

### Results

Both experiments were completed for all 8 dogs. Cardiovascular data were summarized (Figure 1; Table 1). After atipamezole administration in the MED experiment, heart rate (range for all dogs across all time points, 32 to 68 beats/min) was significantly less (*P* < 0.001) than that at baseline (range, 60 to 114 beats/min). At all time points, CI (range, 1.1 to 3.3 L/min/m²) remained significantly less (*P* < 0.001) than baseline (range, 2.3 to 6.7 L/min/m²) and SVRI (range, 5,700 to 23,000 dynes•s/cm²/m²) was significantly greater (*P* < 0.001) than baseline (3,700 to 10,000 dynes•s/cm²/m²). After atipamezole administration in the MEDVAT experiment, cardiovascular function among the 8 dogs was better than that after MED-atipamezole administration; at 45 through 90 minutes after atipamezole administration, heart rate (range, 48 to 102 beats/min) returned to or was greater than the baseline value (55 to 86 beats/min). At all time points, CI (range, 1.9 to 6.1 L/min/m²) was slightly decreased, compared with baseline (2.7 to 6.8 L/min/m²). Between 30 and 90 minutes after atipamezole administration, SVRI (range, 4,100 to 10,000 dynes•s/cm²/m²) was not significantly different from baseline (range, 3,900 to 7,300 dynes•s/cm²/m²). Atipamezole administration decreased MAP transiently in both the MED and MEDVAT experiments. When dogs received

### Table 1—Cardiovascular variables in 8 dogs that received an injection of MED (20 µg/kg) or MED mixed with 400 µg of vatinoxan/kg (MEDVAT treatment) 30 minutes before IM administration of atipamezole (100 µg/kg [given at 0 minutes]) at a 2-week interval in a randomized crossover study.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Treatment</th>
<th>SAP (mm Hg)</th>
<th>DAP (mm Hg)</th>
<th>CVP (mm Hg)</th>
<th>SVI (mL/kg)</th>
<th>RPP (mm Hg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>±10</td>
<td>MED</td>
<td>168 ± 24</td>
<td>83 ± 11</td>
<td>1 ± 2</td>
<td>2.3 ± 0.4</td>
<td>9,750 ± 2,060</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>169 ± 20</td>
<td>80 ± 15</td>
<td>2 ± 2</td>
<td>2.9 ± 0.9</td>
<td>7,500 ± 2,040</td>
</tr>
<tr>
<td>±10</td>
<td>MED</td>
<td>164 ± 20</td>
<td>92 ± 13†</td>
<td>5 ± 2*</td>
<td>1.8 ± 0.6†</td>
<td>4,380 ± 1,360†</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>153 ± 25*</td>
<td>76 ± 10†</td>
<td>6 ± 2*</td>
<td>2.3 ± 1.5*</td>
<td>4,690 ± 1,370*</td>
</tr>
<tr>
<td>5</td>
<td>MED</td>
<td>143 ± 12*</td>
<td>74 ± 10</td>
<td>4 ± 2*</td>
<td>1.7 ± 0.4*</td>
<td>5,140 ± 1,350*</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>132 ± 16*</td>
<td>63 ± 13†‡</td>
<td>3 ± 2*</td>
<td>1.9 ± 0.3*</td>
<td>4,420 ± 806*‡</td>
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<tr>
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<td>MED</td>
<td>148 ± 15*</td>
<td>81 ± 9</td>
<td>4 ± 1*</td>
<td>—</td>
<td>4,700 ± 1,270*</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>134 ± 16*</td>
<td>69 ± 14†‡</td>
<td>3 ± 2*</td>
<td>—</td>
<td>5,250 ± 1,510*</td>
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<tr>
<td>15</td>
<td>MED</td>
<td>146 ± 12*</td>
<td>90 ± 26</td>
<td>4 ± 2*</td>
<td>1.7 ± 0.4*</td>
<td>5,180 ± 1,580*</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>138 ± 16*</td>
<td>73 ± 13†‡</td>
<td>3 ± 2*</td>
<td>1.9 ± 0.3*</td>
<td>5,600 ± 1,880*</td>
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<tr>
<td>20</td>
<td>MED</td>
<td>146 ± 19*</td>
<td>89 ± 11</td>
<td>4 ± 2*</td>
<td>—</td>
<td>5,470 ± 1,560*</td>
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<tr>
<td></td>
<td>MEDVAT</td>
<td>147 ± 12*</td>
<td>86 ± 10</td>
<td>3 ± 2</td>
<td>—</td>
<td>6,860 ± 2,020†</td>
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<tr>
<td>30</td>
<td>MED</td>
<td>144 ± 14*</td>
<td>82 ± 7</td>
<td>4 ± 2*</td>
<td>1.7 ± 0.2*</td>
<td>4,580 ± 652*</td>
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<tr>
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<td>MEDVAT</td>
<td>142 ± 13*</td>
<td>75 ± 11</td>
<td>2 ± 2</td>
<td>2.2 ± 0.4</td>
<td>6,180 ± 1,500†</td>
</tr>
<tr>
<td>45</td>
<td>MED</td>
<td>140 ± 10‡</td>
<td>81 ± 7</td>
<td>4 ± 2*</td>
<td>1.9 ± 0.7*</td>
<td>4,480 ± 832*‡</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>161 ± 11‡</td>
<td>86 ± 13</td>
<td>1 ± 2‡</td>
<td>2.0 ± 0.5*</td>
<td>8,600 ± 2,560†</td>
</tr>
<tr>
<td>60</td>
<td>MED</td>
<td>143 ± 14*</td>
<td>84 ± 7</td>
<td>4 ± 2*</td>
<td>1.9 ± 0.4*</td>
<td>4,320 ± 919*‡</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>155 ± 13*</td>
<td>76 ± 14</td>
<td>1 ± 2†</td>
<td>2.4 ± 0.5</td>
<td>6,670 ± 1,260‡</td>
</tr>
<tr>
<td>90</td>
<td>MED</td>
<td>142 ± 12*</td>
<td>80 ± 9</td>
<td>4 ± 3*</td>
<td>2.0 ± 0.4*</td>
<td>4,010 ± 861*‡</td>
</tr>
<tr>
<td></td>
<td>MEDVAT</td>
<td>162 ± 17†</td>
<td>81 ± 11</td>
<td>1 ± 2‡</td>
<td>2.1 ± 0.5*</td>
<td>8,220 ± 2,120‡</td>
</tr>
</tbody>
</table>

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Data are reported as mean ± SD. For a given treatment, there is a significant (*P* < 0.05) difference from baseline at this time point. †At a given time point, the MEDVAT treatment value is significantly (*P* < 0.05) different from that for the MED treatment.

SVI = Stroke volume index (calculated from data CI and heart rate).

±10 beats/min vs 61 ± 10 beats/min), difference in cardiac output of 1.4 L/min (4.0 ± 1.0 L/min vs 5.4 ± 1.2 L/min), and difference in MAP of 17 mm Hg (110 ± 10 mm Hg vs 95 ± 15 mm Hg).

With regard to changes from baseline at the predetermined time points, differences between treatments were evaluated with repeated-measures ANCOVA models. The models included the main effects of treatment and time point, 2-way interaction of treatment and time point, and a baseline covariate as fixed effects and the main effect of dog, 2-way interaction of dog and time point, and 2-way interaction of dog and treatment as random effects. Composite sedation scores by time point were analyzed nonparametrically with a Wilcoxon signed rank sum test. Normality assumptions were checked with Kolmogorov-Smirnov tests and by assessment of the skewness and kurtosis measures. For DAP, MAP, SAP, RPP, heart rate, blood pH, stroke volume (analyzed and reported as SVI), and cardiac output (analyzed and reported as CI), logarithmic transformation of data was computed to normalize the distribution. With all transformed variables, the model was fitted for changes in the transformed response. However, the descriptive results reported for these variables are in linear scale.

Cardiovascular data were summarized (Figure 1; Table 1). After atipamezole administration in the MED experiment, heart rate (range for all dogs across all time points, 32 to 68 beats/min) was significantly less (*P* < 0.001) than that at baseline (range, 60 to 114 beats/min). At all time points, CI (range, 1.1 to 3.3 L/min/m²) remained significantly less (*P* < 0.001) than baseline (range, 2.3 to 6.7 L/min/m²) and SVRI (range, 5,700 to 23,000 dynes•s/cm²/m²) was significantly greater (*P* < 0.001) than baseline (3,700 to 10,000 dynes•s/cm²/m²). After atipamezole administration in the MEDVAT experiment, cardiovascular function among the 8 dogs was better than that after MED-atipamezole administration; at 45 through 90 minutes after atipamezole administration, heart rate (range, 48 to 102 beats/min) returned to or was greater than the baseline value (55 to 86 beats/min). At all time points, CI (range, 1.9 to 6.1 L/min/m²) was slightly decreased, compared with baseline (2.7 to 6.8 L/min/m²). Between 30 and 90 minutes after atipamezole administration, SVRI (range, 4,100 to 10,000 dynes•s/cm²/m²) was not significantly different from baseline (range, 3,900 to 7,300 dynes•s/cm²/m²). Atipamezole administration decreased MAP transiently in both the MED and MEDVAT experiments.
the MEDVAT treatment, MAP during the first 15 minutes after atipamezole administration (64 to 115 mm Hg) was significantly lower than values during that same period when dogs received the MED treatment (83 to 156 mm Hg). However, hypotension (MAP < 60 mm Hg) was not detected in any dog at any time point.

Data for respiratory rate, selected arterial blood gas variables, and arterial blood pH and lactate concentration were summarized (Table 2). Compared with baseline values, respiratory rate was significantly decreased (P < 0.001) at all time points in both the MED and MEDVAT experiments. Partial pressures of oxygen and carbon dioxide remained stable, with only sporadic differences from baseline. During the 90-minute period after atipamezole administration, arterial blood lactate concentration was higher when dogs received the MED treatment, compared with findings after the dogs received the MEDVAT treatment.

In both experiments, sedation and recovery from sedation were uneventful in all dogs. Composite sedation scores indicated that the dogs became markedly sedated after receiving the MED or MEDVAT treatment (Figure 2). The change in CSS from baseline did not differ significantly between the treatments prior to atipamezole administration. After atipamezole administration in the MEDVAT experiment, there was a steady decrease in CSS over time. In the MED experiment, an initial decrease in CSS was also detected; however, the CSS started to increase again at 30 minutes after atipamezole administration. The

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>RR (breaths/min)</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>Arterial blood pH</th>
<th>Arterial blood lactate concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>–30 MED</td>
<td>27 ± 6</td>
<td>95.6 ± 4.5</td>
<td>35.5 ± 2.3</td>
<td>7.37 ± 0.02</td>
<td>0.56 ± 0.24</td>
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</tr>
<tr>
<td>MEDVAT</td>
<td>28 ± 16</td>
<td>96.8 ± 5.0</td>
<td>35.3 ± 1.9</td>
<td>7.35 ± 0.02</td>
<td>0.49 ± 0.17</td>
<td></td>
</tr>
<tr>
<td>–10 MED</td>
<td>16 ± 8*</td>
<td>94.9 ± 5.1</td>
<td>34.3 ± 2.5</td>
<td>7.36 ± 0.02</td>
<td>0.93 ± 0.37*</td>
<td></td>
</tr>
<tr>
<td>MEDVAT</td>
<td>9 ± 2†</td>
<td>94.0 ± 8.5</td>
<td>32.8 ± 6.3</td>
<td>7.35 ± 0.03</td>
<td>0.73 ± 0.34*</td>
<td></td>
</tr>
<tr>
<td>5 MED</td>
<td>15 ± 3*</td>
<td>101.3 ± 8.1*</td>
<td>34.6 ± 3.3</td>
<td>7.36 ± 0.02</td>
<td>1.40 ± 0.45*</td>
<td></td>
</tr>
<tr>
<td>MEDVAT</td>
<td>10 ± 4†</td>
<td>96.0 ± 5.6†</td>
<td>37.8 ± 2.9</td>
<td>7.33 ± 0.02†</td>
<td>0.90 ± 0.43†</td>
<td></td>
</tr>
<tr>
<td>15 MED</td>
<td>17 ± 4*</td>
<td>99.6 ± 5.2*</td>
<td>35.3 ± 2.3</td>
<td>7.36 ± 0.02†</td>
<td>1.33 ± 0.47*</td>
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<tr>
<td>MEDVAT</td>
<td>18 ± 7*</td>
<td>101.3 ± 7.4*</td>
<td>37.0 ± 2.3</td>
<td>7.34 ± 0.03*</td>
<td>0.95 ± 0.45*</td>
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</tr>
<tr>
<td>30 MED</td>
<td>17 ± 4*</td>
<td>98.4 ± 5.5*</td>
<td>34.9 ± 2.4</td>
<td>7.35 ± 0.02*</td>
<td>1.43 ± 0.48*</td>
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<tr>
<td>MEDVAT</td>
<td>20 ± 4*</td>
<td>98.5 ± 6.4</td>
<td>37.3 ± 1.5</td>
<td>7.33 ± 0.02*</td>
<td>0.79 ± 0.44*</td>
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</tr>
<tr>
<td>45 MED</td>
<td>15 ± 3*</td>
<td>98.6 ± 5.0</td>
<td>35.0 ± 3.0</td>
<td>7.35 ± 0.02*</td>
<td>1.41 ± 0.44*</td>
<td></td>
</tr>
<tr>
<td>MEDVAT</td>
<td>20 ± 3†</td>
<td>98.9 ± 7.1</td>
<td>36.2 ± 1.6</td>
<td>7.34 ± 0.03*</td>
<td>0.80 ± 0.49†</td>
<td></td>
</tr>
<tr>
<td>60 MED</td>
<td>16 ± 5†</td>
<td>100.2 ± 4.8†</td>
<td>34.6 ± 2.5</td>
<td>7.35 ± 0.02*</td>
<td>1.46 ± 0.4*</td>
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<tr>
<td>MEDVAT</td>
<td>22 ± 5†</td>
<td>99.2 ± 6.1</td>
<td>36.1 ± 1.5</td>
<td>7.34 ± 0.03*</td>
<td>0.73 ± 0.44†</td>
<td></td>
</tr>
<tr>
<td>90 MED</td>
<td>15 ± 4*</td>
<td>99.6 ± 4.5*</td>
<td>34.4 ± 2.4</td>
<td>7.37 ± 0.02</td>
<td>1.40 ± 0.48*</td>
<td></td>
</tr>
<tr>
<td>MEDVAT</td>
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<td>98.1 ± 6.2</td>
<td>35.4 ± 2.1</td>
<td>7.35 ± 0.03</td>
<td>0.51 ± 0.29†</td>
<td></td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD.
RR = Respiratory rate
See Table 1 for key.
In the MED experiment, heart rate and CI remained 40% to 60% below baseline, even after atipamezole administration. Vatinox and Vähä-Vähe, which indicate that the initial restoration of heart rate is not sustained and is followed by a subsequent decrease when atipamezole is administered at a dose 2 to 4 times the preceding dose of MED. It has also been shown that atipamezole does not restore heart rate to presedation level in dogs treated with MED, although substantially, clinically relevant, and more sustained increases in heart rate after atipamezole administration have also been reported.1,4,8 Hence, the capacity of atipamezole to reverse the bradycardia attributed to α2-adrenoceptor agonists may be variable. In dogs, even very low doses of dexmedetomidine induce peripherally and centrally mediated bradycardia27 and also provide anxiolysis without sedative effect.28 This could explain why heart rate remains less than presedation values in MED-treated dogs even after reversal of sedation with atipamezole.

In the present study, atipamezole failed to restore SVRI or increase CI to baseline values when dogs received the MED treatment. Throughout the observational period after atipamezole administration, SVRI was doubled and CI remained depressed, compared with the respective baseline values. Thus, atipamezole was not able to effectively reverse the vasoconstrictive and bradycardic effects of MED. In a previous study3 of dogs wherein atipamezole (97.5 to 115 µg/kg) was administered IM 60 minutes after treatment with MED (39 to 46 µg/kg) that was combined with midazolam and butorphanol, buprenorphine, or saline solution, atipamezole (at a dose 2.5 times that of MED) was able to abolish the cardiovascular and respiratory effects of MED, although CI failed to return to baseline. In another study29 in which atipamezole (80 µg/kg) was administered IM 40 minutes after treatment with MED (20 µg/kg) that was combined with midazolam, atipamezole (at a dose 4 times that of MED) effectively and rapidly decreased systemic vascular resistance and increased MED-depressed heart rate and CI, both of which reached baseline values within 20 minutes. Similarly, atipamezole completely reversed all cardiovascular changes attributed to dexmedetomidine when atipamezole was administered IV at a high dose of 300 to 500 µg/kg.30,31 The results of previous studies are not fully comparable to those of the present study because of the differences in study designs. In a study by Pypendop et al,5 the dose of MED was double that used in the present study, and MED was combined with midazolam and an opioid; moreover, atipamezole was administered at a dose half that used in the present study and given 60 minutes after agonist administration. The protocol of Hayashi et al29 was more similar to that of the present study, although those researchers used MED that was combined with midazolam and the atipamezole dose was 20% lower and given 10 minutes later than the atipamezole dose used in the present study. All of the aforementioned data indicate that the dose of atipamezole and route and timing of its administration appear to influence the cardiovascular outcome of such treatment.

**Figure 3**—Mean ± SD plasma dexmedetomidine concentrations in the dogs in Figure 1 during the MED (squares) and MEDVAT (circles) experiments. A venous blood sample was collected from each dog before (~10 minutes) and after (5, 10, 15, 20, 30, 60, 90, and 210 minutes) atipamezole administration at 0 minutes (arrow) for analysis. Error bars indicate the 95% confidence intervals of the means. See Figure 1 for remainder of key.
There are several possible reasons for the differences in the cardiovascular findings of the present study, compared with those of previous studies, namely differences in the drugs, doses, and routes and timings of administration used; differences in the methods of cardiovascular variable measurement (eg, ECG measurement of heart rate regardless of possible drug-induced changes in P waves and QRS complexes or measurement of cardiac output with a thermodilution vs lithium dilution method); and differences in the dogs’ temperament and reactions to handling, monitoring, mensuration, and surroundings, which affect their activity and cardiovascular status during collection of data at baseline and during the recovery period. In the present study, dogs were gently restrained on an examination table until 90 minutes after atipamezole administration. Baseline values in the present study were comparable to those of previous studies but lack of physical activity and residual sedation might have affected the dogs’ cardiovascular function during recovery from sedation. Hence, further studies with larger numbers of dogs and that better represent a clinical setting are still required.

Although some of the discordant findings between previous investigations and the present study may be difficult to reconcile; results of the present study indicated that cardiovascular function in dogs was improved when vatinoxan was coadministered with MED, both before and after reversal of sedation with atipamezole. With the MEDVAT treatment, heart rate, CI, RPP, and SVRI returned to or very close to reference values. Medetomidine induces cardiovascular changes both peripherally and centrally by causing vasoconstriction and baroreflex-mediated bradycardia and diminishing sympathetic tone. Vatinoxan affects only the peripheral organs, preserving cardiovascular functions in dexmedetomidine- and MED-sedated dogs whereas atipamezole also reverses the central effects of α₂-adrenoceptor agonists. In the dogs of the present study, atipamezole and vatinoxan appeared to interact in a favorable manner, although momentary decreases in arterial blood pressure attributable to α₂-adrenoceptor blockade were noticed shortly after atipamezole administration. Similar initial decreases in arterial blood pressure after IM administration of atipamezole are a result of antagonism of the dexmedetomidine- and MED-mediated peripheral vasoconstriction have been found in other studies. In the present study, the magnitude of the momentary decrease in MAP was similar whether dogs did or did not receive vatinoxan. Because MAP was lower after MEDVAT administration than it was after MED administration at the -10-minute time point (ie, before atipamezole administration), the lowest MAP was detected when dogs received the MEDVAT treatment. Notably, no clinically relevant hypotension was detected in any dog at any time during the experiments in the present study.

A decrease in respiratory rate was evident in dogs following both treatments in the present study, and contrary to previous reports, atipamezole administration did not significantly increase respiratory rate. However, the gentle restraint of dogs on an examination table during recovery from sedation might have influenced their breathing. Nevertheless, PaO₂, PaCO₂, and arterial blood lactate concentration and pH remained within reference ranges throughout the MED and MEDVAT experiments, although the comparatively higher arterial blood lactate concentration following MED administration suggested more pronounced compromise of tissue perfusion with that treatment. The limited published data available suggest that the decrease in cardiac output attributed to α₂-adrenoceptor agonists does not cause detrimental hypoperfusion of vital organs in healthy dogs, but intestinal and skeletal blood flows are decreased. These findings might explain the higher arterial blood lactate concentration when the dogs received the MED treatment because vatinoxan improves perfusion in peripheral tissues.

The dogs’ recoveries from sedation after atipamezole administration were calm and smooth with both treatments. However, dogs treated with MED without vatinoxan tended to relapse into sedation. Resedation was not noticed in dogs treated with MEDVAT. Similar to our findings, Vainio and Vähä-Vahē reported drowsiness after atipamezole administration in almost half of the medetomidine-sedated dogs. It was described that the dogs in that study could stand up and walk if forced to do so, but they preferred to lay down and were somnolent. Resedation or prolonged sedation after atipamezole administration has, nevertheless, been described as rare in dogs, but it is common in ruminants. Administration of vatinoxan appeared to ensure more complete recovery from sedation when atipamezole was used to reverse the effects of MED in sheep, which is in line with the finding for dogs of the present study. At the time of resedation, plasma dexmedetomidine concentrations when dogs received the MED treatment were significantly higher than those detected when dogs received the MEDVAT treatment. Because atipamezole is a competitive α₂-adrenoceptor antagonist, high plasma agonist concentration would be expected to impair the desired antagonistic effect of atipamezole administration. Compared with the effects of the MED treatment in the dogs of the present study, coadministration of vatinoxan with MED maintained higher cardiac output (as evidenced by the higher CI values), thereby providing better hepatic perfusion and increasing the plasma clearance of dexmedetomidine; thus, more complete recoveries from sedation were facilitated.

Administration of vatinoxan with MED accelerates MED absorption after IM injection, as was apparent in the present study wherein peak plasma concentrations of dexmedetomidine in the dogs were observed earlier after MEDVAT treatment than they were after MED treatment. Vatinoxan is absorbed more slowly than MED when the drugs are concomitantly given IM.
Therefore, early cardiovascular effects of MED were not prevented by vatinoxan, and an initial increase in SVRI coupled with decreases in heart rate and CI was also evident after MEDVAT administration, although to a lesser extent than those associated with the MED treatment. Compared with findings in the MED experiment, coadministration of vatinoxan with MED decreased dexmedetomidine concentrations faster in plasma after atipamezole administration, as presumed on the basis of results of a previous study, which indicated that administration of vatinoxan significantly increased the plasma clearance of dexmedetomidine overall. In the dogs of the present study, both $\alpha_2$-adrenoceptor antagonists (vatinoxan and atipamezole) probably hastened the elimination of MED as indicated by enhanced hemodynamic function and faster and more complete recovery from sedation after atipamezole administration, even though the plasma exposure to atipamezole was reduced by vatinoxan.

In the present study, administration of atipamezole alone failed to result in sustained decreases in SVRI or increases in heart rate and CI in MED-sedated dogs, and those dogs relapsed into sedation after initial arousal was observed. However, when dogs received the MEDVAT treatment, atipamezole was able to reverse the sedative effect of MED more efficiently and reduce the depression of cardiovascular function. When atipamezole was used for reversal of MEDVAT sedation in dogs, the $\alpha_2$-adrenoceptor antagonists interacted favorably, without adverse effects. Hence, coadministration of the peripherally acting $\alpha_2$-adrenoceptor antagonist vatinoxan with MED might enhance the quality and safety of recovery from MED-induced sedation in dogs receiving atipamezole as reversal agent.

Acknowledgments
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Presented in abstract form at the 12th World Congress of Veterinary Anaesthesiology, Kyoto, Japan, September 2015 and at the autumn meeting of the Association of Veterinary Anesthetists, Berlin, Germany, November 2017.

Footnotes
b. Dorbene (1 mg/mL), Laboratorios SYVA S.A.U., León, Spain.
c. Vetcare Ltd, Mäntsälä, Finland.
d. Alzane (5 mg/mL), Laboratorios SYVA S.A.U., León, Spain.
f. Arteriofix V, B. Braun Melsungen AG, Berlin, Germany.
g. CV-12702, Arrow International, Reading, Pa.
h. Gabarith PMSET, Becton Dickinson, Sandy, Utah.
i. S/5 Anesthesia Monitor, GE Healthcare, Helsinki, Finland.
k. Lithium chloride (0.15 mmol/mL) solution for injection, LiDCO Ltd, London, England.
l. ABL 855, Radiometer, Copenhagen, Denmark.
m. Sep-Pak C18, 96-well extraction plates, Waters Co, Milford, Mass.
n. Toronto Research Chemicals Inc, Toronto, ON, Canada.
o. RS-79948, Tocris Bioscience, Bristol, England.
p. SunFire C18 column (2.1 X 150 mm; pore size, 3.5 µm), Waters Co, Milford, Mass.
q. Chiralpak AGP column (4 X 150 mm; pore size, 5 µm), Chiral Technologies Europe, Illkirch-Graffenstaden, France.
r. 4000QTrap, MDS Sciex, Concord, ON, Canada.
s. Waters Acquity UPLC and Waters TQ-S triple quadrupole mass spectrometry, Waters Co, Milford, Mass.

References
15. Restitutti F, Honkavaara JM, Raekallio MR, et al. Effects of...


Appendix appears on the next page
Appendix

Modified composite sedation scoring system (0 to 20 points) used to assess sedation in dogs.

<table>
<thead>
<tr>
<th>Assessment (possible scores)</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Position (0–4)</td>
<td>Dog is standing</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dog is standing but staggers</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dog is in sternal recumbency with its head up</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Dog is in sternal recumbency with its head down</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Dog is in lateral recumbency with its head down</td>
<td>4</td>
</tr>
<tr>
<td>Palpebral reflex (0–3)</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Slightly reduced</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Weak</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>3</td>
</tr>
<tr>
<td>Eye position (0 or 2)</td>
<td>Central</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Turned down</td>
<td>2</td>
</tr>
<tr>
<td>Jaw and tongue relaxation (0–4)</td>
<td>Normal resistance on opening the dog’s mouth and manipulation of the tongue</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dog closes jaws together</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dog opens the jaws but there is strong resistance when the tongue is pulled</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Dog opens the jaws but there is slight resistance when the tongue is pulled</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Dog shows no resistance to jaw opening or tongue pulling</td>
<td>4</td>
</tr>
<tr>
<td>Resistance to positioning in lateral recumbency (0–3)</td>
<td>Dog struggles normally or resists this positioning</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dog returns to sternal recumbency</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Some resistance but the dog remains in lateral recumbency</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>No resistance or the dog is already in lateral recumbency</td>
<td>3</td>
</tr>
<tr>
<td>General appearance (0–4)</td>
<td>Dog is awake with a normal reaction to surroundings</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Dog appears slightly tired and its head is drooping</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Signs of mild sedation; dog clearly reacts to surroundings</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Signs of moderate sedation; dog reacts slightly to surroundings</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Signs of deep sedation; dog has no reaction to surroundings</td>
<td>4</td>
</tr>
</tbody>
</table>
Effects of intramuscular vatinoxan (MK-467) coadministered with medetomidine and butorphanol on the cardiopulmonary and anaesthetic effects of intravenous ketamine in dogs

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ABSTRACT
Objective
To investigate the impact of intramuscular (IM) coadministration of the peripheral α2-adrenoceptor agonist vatinoxan (MK-467) with medetomidine and butorphanol prior to intravenous (IV) ketamine on the cardiopulmonary and anaesthetic effects in dogs, followed by atipamezole reversal.

Study design
Randomized masked crossover study.

Animals
A total of eight purpose-bred, 3-year-old Beagle dogs.

Methods
Each dog was instrumented and administered two treatments, 2 weeks apart: medetomidine (20 µg kg⁻¹) and butorphanol (100 µg kg⁻¹) premedication with vatinoxan (500 µg kg⁻¹; treatment MVB) or without vatinoxan (treatment MB) IM 20 minutes before IV ketamine (4 mg kg⁻¹). Atipamezole (100 µg kg⁻¹) was administered IM 60 minutes after ketamine. Heart rate (HR), mean arterial (MAP) and central venous (CVP) pressures and cardiac output (CO) were measured and cardiac (CI) and systemic vascular resistance (SVRI) indices were calculated before and 10 minutes after MVB or MB, and 10, 25, 40, 55, 70 and 100 minutes after ketamine. Data were analyzed with repeated measures analysis of covariance models. p-values <0.05 were considered statistically significant. Sedation, induction, intubation and recovery scores were assessed.

Results
At most time points HR and CI were significantly higher and SVRI and CVP significantly lower with MVB than MB. With both treatments, SVRI and MAP decreased after ketamine, whereas HR and CI increased. MAP was significantly lower with MVB than MB; mild hypotension (57-59 mmHg) was recorded in two dogs with MVB prior to atipamezole administration. Sedation, induction, intubation and recovery scores were not different between treatments, but intolerance to the endotracheal tube was observed earlier with MVB.

Conclusions and clinical relevance
Haemodynamic performance was improved by coadministration of vatinoxan with medetomidine-butorphanol, before and after ketamine administration. However, vatinoxan was associated with mild hypotension after ketamine with the dose used in this study. Vatinoxan shortened the duration of anaesthesia.

Keywords anaesthesia, butorphanol, cardiopulmonary, ketamine, medetomidine, vatinoxan

Introduction
α2-adrenoceptor agonists, such as medetomidine or its pharmacologically active enantiomer dexmedetomidine, can be combined with butorphanol resulting in reliable sedation, muscle relaxation and analgesia for clinical procedures (Bartram et al. 1994; Ko et al. 2000; Kuo & Keegan 2004; Leppänen et al. 2006). Cardiovascular depression characterized by increased systemic vascular resistance index (SVRI) and decreased heart rate (HR) and cardiac index (CI), attributed to α2-adrenoceptor agonist-induced peripheral vasoconstriction and decrease in sympathetic outflow, limit the usefulness of such combinations (Bartram et al. 1994; Pypendop & Verstegen 1998; Kuo & Keegan 2004).

To reduce these adverse cardiovascular effects in dogs, a peripheral α2-adrenoceptor antagonist, vatinoxan (also known as MK-467 and L-659066) has been tested for concomitant use with medetomidine. The direct influence of vatinoxan is restricted to peripheral tissues because of its limited penetration across the mammalian blood-brain barrier (Clinkesmidt et al. 1988). Therefore, vatinoxan does
not markedly counteract medetomidine-induced sedation (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012) but helps to maintain cardiac output (CO) by preventing the arterial vasconstrictive effects of $\alpha_2$-adrenoeceptor agonists (Piascik et al. 1996; Pagel et al. 1998; Enouri et al. 2008; Honkavaara et al. 2011). Even if the initial haemodynamic effects of medetomidine, are not entirely prevented by intramuscularly (IM) coadministered vatinoxan, their intensity and duration are reduced (Restitutti et al. 2017). Similarly, when IM medetomidine-vatinoxan is further combined with butorphanol, lesser reductions in HR (Salla et al. 2014; Kallio-Kujala et al. 2018) and CI have been observed (Salla et al. 2014).

Ketamine, a N-methyl-D-aspartate receptor antagonist (NMDA), is a dissociative anaesthetic often used for induction of anaesthesia in dogs. The advantages of premedication with an $\alpha_2$-adrenoeceptor agonist prior to ketamine induction include a dose-sparing effect and predictable, rapid and smooth induction of anaesthesia followed by short-lasting surgical anaesthesia for mildly painful procedures (Hellebrekers & Sap 1997; Hellebrekers et al. 1998). Post-procedural recovery may be hastened with the use of an $\alpha_2$-adrenoeceptor antagonist, such as atipamezole (Ko et al. 2000). Anaesthetic doses of ketamine are often associated with transient respiratory depression that may be intensified by $\alpha_2$-adrenoeceptor agonists and opioids (Ko et al. 2001; Barletta et al. 2011; Krimins et al. 2012). Ketamine has sympathomimetic effects, stimulating cardiovascular function by increasing HR, CO and arterial blood pressure (Haskins et al. 1985). However, ketamine may also have direct negative inotropic effects (Pagel et al. 1992).

Ketamine has been investigated for intravenous (IV) anaesthetic induction in dogs sedated with medetomidine and vatinoxan (Salla et al. 2017), but the dose of ketamine administered was small (1 mg kg$^{-1}$) and no opioid agent was used. In the present study, the primary aim was to evaluate the influence of vatinoxan on cardiopulmonary function and anaesthetic effects in dogs administered medetomidine-butorphanol IM 20 minutes before induction of anaesthesia with a higher IV dose of ketamine (4 mg kg$^{-1}$). Secondly, recovery characteristics before and after atipamezole administration were assessed. Atipamezole interacts favorably with vatinoxan when used to reverse medetomidine-induced sedation (Turunen et al. 2019). However, there are no reports of the interaction of atipamezole and vatinoxan with ketamine.

We hypothesized that 1) vatinoxan would improve cardiovascular performance by decreasing SVRI and increasing HR and CI; 2) by preserving CO, vatinoxan would hasten the plasma clearance of ketamine and shorten the duration of anaesthesia; and 3) atipamezole administered 60 minutes after ketamine would shorten recovery without detrimental adverse effects. We did not expect any impact of vatinoxan on ventilation, clinical quality of induction or intubation between treatments.

### Materials and methods

#### Animals

A total of eight purpose-bred Beagle dogs (six neutered males and two neutered females) approximate age 3 years, weighing 13.3 ± 1.7 kg [mean ± standard deviation (SD)] were studied. The dogs were considered healthy based on history and comprehensive clinical examinations including complete blood counts and routine serum chemistry. The dogs were housed in groups in a kennel, fed with commercial food with free access to water. Food was withheld for 12 hours prior to experiments. The study was approved by Finnish Animal experimental board (ESAVI/7187/04.10.03/2012). All dogs have been rehomed.

#### Treatments

Each dog was administered two treatments in a randomized (www.random.org) crossover design, separated by ≥2 weeks. Dogs in treatment MVB were premedicated IM with medetomidine hydrochloride (20 µg kg$^{-1}$; Dorbene, 1 mg mL$^{-1}$; Laboratorios SYVA S.A.U., Leon, Spain), butorphanol tartrate (100 µg kg$^{-1}$; Torpudor vet, 10 mg mL$^{-1}$; Richter Pharma AG, Wels, Austria) and vatinoxan hydrochloride (500 µg kg$^{-1}$; Recipharm, Umea, Sweden). Premedication in treatment MB was medetomidine and butorphanol at the same dose rates. Drugs were administered from one syringe 20 minutes before IV ketamine (4 mg kg$^{-1}$; Ketaminol, 50 mg mL$^{-1}$; Intervet International B.V., Boxmeer, The Netherlands). Atipamezole hydrochloride (100 µg kg$^{-1}$; Alzane, 5 mg mL$^{-1}$; Laboratorites SYVA S.A.U.) was administered IM 60 minutes after ketamine.

The medetomidine-vatinoxan solution was prepared immediately before use in a sterile vial containing 25 mg of vatinoxan powder, by adding 1 mL of medetomidine and 1 mL of physiological saline solution (Natriumklorid 0.9 %; B. Braun, Germany) and mixing the vial until the solution was clear by visual inspection. The final drug concentrations in the solution were 500 µg mL$^{-1}$ of medetomidine and 12.5 mg mL$^{-1}$ of vatinoxan (ratio of 1:25). The medetomidine solution was prepared similarly, in an empty vial. An injection volume of 0.04 mL kg$^{-1}$ was drawn from the vial and butorphanol was added to the same syringe.

#### Instrumentation

Prior to each experiment, each dog was instrumented under general anesthesia. A cephalic vein was aseptically cannulated with a 22 gauge catheter for induction of anaesthesia using propofol (6.3-14.6 mg kg$^{-1}$; Vetofol vet, 10 mg mL$^{-1}$; Norbrook Laboratories, Monaghan, Ireland) administered IV to effect. The dog was then intubated orotracheally. Anaesthesia was maintained with isoflurane (Vertflurane 1000 mg g$^{-1}$; Virbac S.A., Carros, France) in oxygen delivered via a circle breathing system (Anesco Inc, FL, USA) maintaining the end-tidal isoflurane concentration (FE $\text{ISO}$) at 1.5%. Mechanical ventilation was applied to maintain the end-tidal carbon dioxide partial pressure at 35–45 mmHg (4.7–6.0 kPa). Blood pressure, electrocardiography and pulse oximetry were
Arterial blood samples were anaerobically collected for and blood pressures were recorded first; at confirmed by auscultation over 1 minute), respiratory treatments. confirm extravascular drug administration for all IM the left pelvic limb 60 minutes after ketamine.

peak inspiratory pressure of 10 ventilation), th

via a circle breathing system (ketamine bolus, oxygen at 1 L minute

administered minutes after premedication, ketamine was

into the gluteal muscles of the right pelvic limb. At 20

blood samples,

Immediately after obtaining all baseline values and

pressures and a continuous lead II electrocardiogram

electrocardiography.

both thoracic limbs and the left pelvic limb for

pressure levels than is considered physiological for

Finland (Dickinson, UH, USA)

arterial and central venous catheters were connected to

i

The dog was minimally restrained in sternal

foam pa

from anaesthesia. At

. The ac

accuracy of the transducers was verified

before each experiment and zeroed to

a mercury manometer (counting chest movements during 1 minute)

R

1

1

-1

-1

20 mg mL

-1

Orion Pharma, Espoo, Finland

-2

12702; Arrow

-1

was aseptically inserted into a

-1

jugular vein under local anaesthesia (0.25 mL. of Lidocain 20 mg mL

-1

introduced to a premeasured distance for the tip of the

catheter to reach the cranial border of the second rib at the costochondral junction and secured with sutures and a light bandage. Then delivery of isoflurane and IV fluid were discontinued, and the dog was allowed to recover from anaesthesia. At least 60 minutes elapsed after extubation prior to baseline measurements.

Measurements

The dog was minimally restrained in sternal recumbency on an examination table covered with an insulating foam pad and electrical heating pad. The arterial and central venous catheters were connected to pressure transducers (Gabarith PMSET; Becton Dickinson, UH, USA) with saline filled pressure tubing (Argon Medical Devices Netherlands B.V., Helsinki, Finland). The accuracy of the transducers was verified with a mercury manometer at higher and lower pressure levels than is considered physiological for dogs before each experiment and zeroed to atmospheric pressure at the level of the manubrium. Adhesive electrodes were placed on shaved skin of both thoracic limbs and the left pelvic limb for electrocardiography. Invasive arterial and venous pressures and a continuous lead II electrocardiogram (ECG) were monitored (S/5 Anesthesia Monitor; GE Healthcare, Helsinki, Finland) throughout the session. Immediately after obtaining all baseline values and blood samples, the assigned treatment was injected into the gluteal muscles of the right pelvic limb. At 20 minutes after premedication, ketamine was administered IV over 2 minutes. Orotracheal intubation was performed immediately after the ketamine bolus, oxygen at 1 L minute

-1

delivered via a circle breathing system (Anesco Inc, FL, USA). If apnoea occurred (i.e. 30 seconds without spontaneous ventilation), the dog was manually ventilated with a peak inspiratory pressure of 10-15 cm H

2

O

2-3 times minute

-1

until spontaneous breathing resumed. Atipamezole was injected into the gluteal muscles of the left pelvic limb 60 minutes after ketamine. Negative aspiration for blood was performed to confirm extravascular drug administration for all IM treatments.

HR (recorded from the ECG and confirmed by auscultation over 1 minute), respiratory rate (f

R

(10, 15, 25, 40, 55, 70 and 100) and 120 for HR) minutes from administration of ketamine. Arterial blood samples were anaerobically collected prior to CO measurements into heparinized syringes (Pico50; Radiometer Medical ApS, Copenhagen, Denmark) and analyzed immediately for pH, arterial partial pressures of carbon dioxide (PaCO

2

and oxygen (PaO

2

), lactate, sodium and haemoglobin concentrations (ABL 855; Radiometer Medical ApS), corrected to individual rectal temperature. CO was measured with the lithium dilution method (LiDCO Plus hemodynamic monitor; LiDCO Ltd, UK) with a standard dose of 0.075 mmol of lithium chloride (LiDCO Ltd) injected via the central venous catheter (Mason et al. 2001) at -20 (baseline), -10, 10, 25, 40, 55, 70 and 100 minutes from the administration of ketamine. Initial standard values of 10 g dL

-1

for haemoglobin and 140 mmol L

-1

for sodium were later corrected with actual values obtained from arterial blood. CI, stroke volume index (SVI), rate pressure product (RPP) and SVRI were calculated by using standard equations (Haskins et al. 2005).

Venous blood samples (6 mL into EDTA tubes) were collected from the central venous catheter at 10, 40, 55 and 70 minutes for plasma concentration analyses of studied drugs (dexametomidine, butorphanol, ketamine, vatinoxan, atipamezole). Samples were separated by refrigerated centrifugation (4 °C 2520 g for 15 minutes), and plasma was kept frozen at -20°C or colder until analyzed. Purpose of the drug concentration analysis was to detect the influence of vatinoxan on plasma concentrations of coadministered drugs, and compare it to clinical observations.

Composite sedation scores (ranging from 0, representing no sedation, to 20, considered as deep sedation) were determined after recording of HR. Induction scores (ideal, good, unsatisfactory, not reached), jaw tone (poor, slight, good, total) and intubation scores (smooth, mild coughing, pronounced coughing, swallowing or gagging, failed attempt) were recorded immediately after successful endotracheal intubation. The early recovery score (easy or fairly easy transition to alertness, restless, needs restraint) was assessed after extubation and the late recovery score (alert and responsive, slightly sedated, sedated, very sedated) 120 minutes after ketamine administration. An investigator (HT) unaware of the treatment and the cardiovascular variables assigned all scores. Times from administration of premedication to sedation, defined as the head resting on the table, and to return of consciousness were recorded. The time when the dog coughed or swallowed around the endotracheal tube resulting in extubation and any adverse clinical signs during recovery, such as nausea, were recorded. After recovery from anaesthesia, the catheters were removed and meloxicam (0.2 mg kg

-1

Metacam, 5 mg mL

-1

Boehringer Ingelheim Vetmedica GmbH, Rhein, Germany) was administered subcutaneously. The dog was offered food prior to returning to its kennel.
**Analytical methods**

Concentrations of dexmedetomidine in canine plasma were determined with chiral high performance liquid chromatography-mass spectrometry (HPLC-MS/MS, Sciex API 4000, ON, Canada) as described previously (Adam et al. 2018). Reference samples were prepared in drug-free canine plasma. The precursor ion–fragment ion pairs monitored were m/z 201.2–95.1 for dexmedetomidine and m/z 204.2–98.1 for the internal standard, deuterated (D₃) medetomidine. The accuracy of the quality control samples (at levels of 0.225, 1.0 and 8.0 ng mL⁻¹) ranged from 91.3% to 99.1%, and intra-assay CV% at these concentration levels ranged from 1.2% to 3.9% for dexmedetomidine.

The concentrations of butorphanol, ketamine, atipamezole and vatinoxan in plasma were analyzed with HPLC-MS/MS (Waters Acquity UPLC + Waters TQ-S triple quadrupole MS; Waters Co., MA, USA) as described previously (Kallio-Kujala et al. 2018). The selected reaction monitoring was m/z 1 of 328 > 124 for butorphanol, m/z 1 of 238 > 220 for ketamine, m/z 1 of 213 > 117 for atipamezole, m/z 1 of 419 > 200 for vatinoxan and m/z 1 of 260 > 116 for the employed internal standard propranolol. Results of all quality control samples were within 85-115% of their nominal concentration.

**Statistical methods**

The sample size calculations were based on data (mean ± SD) from previous studies (Honkavaara et al. 2008, 2011; Restitutti et al. 2017). Eight dogs were used to detect following differences (peak effects) between treatments (paired two-tailed test, α-level 0.05, power 80%): HR 11 beats minute⁻¹ (50 ± 10 versus 61 ± 10 beats minute⁻¹), CO 1.4 L minute⁻¹ (4.0 ± 1.0 versus 5.4 ± 1.2 L minute⁻¹), mean arterial pressure (MAP) 17 mmHg (110 ± 10 versus 93 ± 15 mmHg).

Differences in several responses within and between treatments were evaluated with repeated measures analysis of covariance models. For between comparisons, actual values were used as response in the model, and for within treatment comparisons change from baseline was used. The models included the main effects of treatment and time point, two-way-interaction of treatment and time point, and a baseline covariate as fixed effects, and the main effect of dog, the two-way-interactions of dog and time point and dog and treatment as random effects. Additionally, the values after ketamine and atipamezole were evaluated including only time points after administration, and the values prior ketamine and atipamezole administration were used as baseline covariates. With continuous variables measured only once, depending on their distribution, either paired t tests or Wilcoxon’s signed rank sum test were used. Differences in occurrence of nausea (yes or no) were evaluated with McNemar’s test. Normality assumptions were checked with Kolmogorov–Smirnov test. In case normality assumptions were not met, common transformations were used (logarithm, inverse). Estimates of treatment differences were calculated over time and by time point with contrasts from the fitted models. Similarly, within-group changes were calculated for each time point with contrasts from the fitted models. Differences in plasma drug concentrations and their areas under the curve (AUC) were compared with paired, two-tailed t tests. For the group differences and within-group changes 95% confidence intervals and p-values were calculated. p-values by time point were adjusted using the Bonferroni correction within each model (and treatment) and p-values <0.05 were considered statistically significant. SAS for Windows, Version 9.3 (SAS Institute Inc.; NC, USA) was used for all statistical analyses.
Figure 1 - Mean ± standard deviation of (a) heart rate, (b) cardiac index, (c) mean arterial pressure and (d) systemic vascular resistance index of eight dogs administered intramuscular (IM) premedication with medetomidine (20 µg kg\(^{-1}\); treatment MB; open squares) or medetomidine and butorphanol at the same dose rates with vatoxan (500 µg kg\(^{-1}\); treatment MVB; solid circles) 20 minutes before intravenous ketamine (4 mg kg\(^{-1}\); \(\times\)) and 80 minutes before IM atipamezole (100 µg kg\(^{-1}\); \(\odot\)). * Significant difference between treatments (p < 0.05). †Significantly different from –20 minutes within the treatment (p < 0.05). ‡Significantly different from –10 minutes within the treatment (p < 0.05). §Significantly different from 55 minutes within a treatment (p < 0.05).

Table 1 - Cardiovascular variables in eight dogs that were administered intramuscularly (IM) medetomidine (20 µg kg\(^{-1}\)) and butorphanol (100 µg kg\(^{-1}\); treatment MB) or medetomidine (20 µg kg\(^{-1}\)), vatoxan (500 µg kg\(^{-1}\)) and butorphanol (100 µg kg\(^{-1}\); treatment MVB) immediately after baseline measurements at time -20. Intravenous injection of ketamine (4 mg kg\(^{-1}\)) was administered at time 0. Intramuscular injection of atipamezole (100 µg kg\(^{-1}\)) was administered at time 60. Values are mean ± standard deviation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Time (minutes)</th>
<th>20</th>
<th>10</th>
<th>15</th>
<th>25</th>
<th>40</th>
<th>55</th>
<th>70</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP (mmHg)</td>
<td>MB</td>
<td>163 ± 28</td>
<td>183 ± 20(\dagger)</td>
<td>159 ± 17</td>
<td>152 ± 12(\dagger)</td>
<td>145 ± 15(\dagger)</td>
<td>139 ± 13(\dagger)</td>
<td>154 ± 13(\dagger)</td>
<td>143 ± 23(\dagger)</td>
<td>147 ± 15</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>MVB</td>
<td>164 ± 14</td>
<td>154 ± 14(\dagger)</td>
<td>107 ± 16(\dagger)</td>
<td>107 ± 13(\dagger)</td>
<td>106 ± 12(\dagger)</td>
<td>105 ± 11(\dagger)</td>
<td>107 ± 9(\dagger)</td>
<td>148 ± 23(\dagger)</td>
<td>150 ± 16(\dagger)</td>
</tr>
<tr>
<td>CVP (mmHg)</td>
<td>MB</td>
<td>80 ± 7</td>
<td>108 ± 5(\dagger)</td>
<td>106 ± 7(\dagger)</td>
<td>93 ± 8</td>
<td>78 ± 10(\dagger)</td>
<td>73 ± 10(\dagger)</td>
<td>70 ± 8(\dagger)</td>
<td>75 ± 14(\dagger)</td>
<td>76 ± 10(\dagger)</td>
</tr>
<tr>
<td>CVI (cm (\times) min (^{-1}))</td>
<td>MVB</td>
<td>84 ± 9</td>
<td>91 ± 38(\dagger)</td>
<td>59 ± 8(\dagger)</td>
<td>54 ± 9(\dagger)</td>
<td>52 ± 10(\dagger)</td>
<td>51 ± 8(\dagger)</td>
<td>49 ± 8(\dagger)</td>
<td>72 ± 14(\dagger)</td>
<td>72 ± 11(\dagger)</td>
</tr>
<tr>
<td>RPP (mmHg (\times) min (^{-1}))</td>
<td>MB</td>
<td>2.0 ± 0.3</td>
<td>1.4 ± 0.2(\dagger)</td>
<td>1.4 ± 0.3(\dagger)</td>
<td>1.8 ± 0.4</td>
<td>1.9 ± 0.3(\dagger)</td>
<td>1.8 ± 0.6</td>
<td>2.7 ± 0.3(\dagger)</td>
<td>2.1 ± 0.3(\dagger)</td>
<td>2.1 ± 0.3(\dagger)</td>
</tr>
<tr>
<td>RPP (mmHg (\times) min (^{-1}))</td>
<td>MVB</td>
<td>1.8 ± 0.3</td>
<td>1.8 ± 0.3</td>
<td>1.7 ± 0.4</td>
<td>1.6 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>1.9 ± 0.4</td>
<td>2.0 ± 0.6(\dagger)</td>
<td>2.2 ± 0.3</td>
<td>2.2 ± 0.3</td>
</tr>
</tbody>
</table>

SAP, systolic arterial pressure; DAP, diastolic arterial pressure; CVP, central venous pressure; SVI, stroke volume index; RPP, rate pressure product.

\(\dagger\) Significant difference from MB at the same time (p < 0.05). †Significantly different from time -20 within the same treatment (p < 0.05). ‡Significantly different from time -10 within the same treatment (p < 0.05). §Significantly different from time 55 within the same treatment (p < 0.05).
Table 2 - Mean ± standard deviation of respiratory rate (fR), arterial partial pressure of carbon dioxide (PaCO2), arterial pH (pHa), lactate concentration and rectal temperature (RT) in eight dogs. Variables were recorded before drug administration (time –20), 10 minutes after premedication (time –10) with medetomidine (20 µg kg\(^{-1}\)) and butorphanol (100 µg kg\(^{-1}\); treatment MB; open squares) or medetomidine and butorphanol at the same dose rates with vatinoxan (500 µg kg\(^{-1}\); treatment MVB; solid circles) 20 minutes before intravenous ketamine (4 mg kg\(^{-1}\); ○) and 80 minutes before IM atipamezole (100 µg kg\(^{-1}\); ●). *Significantly different from MB at the same time (p < 0.05); † Significantly different from time –20 within the treatment (p < 0.05); ‡ Significantly different from time –10 within the treatment (p < 0.05); § Significantly different from time 55 (p < 0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fR</td>
<td>MB</td>
<td>24 ± 6 13 ± 3(^{†})</td>
</tr>
<tr>
<td></td>
<td>MVB</td>
<td>24 ± 6 13 ± 3(^{†})</td>
</tr>
<tr>
<td>PaCO(_2) mmHg</td>
<td>MB</td>
<td>35.3 ± 3.0 35.3 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>MVB</td>
<td>36.4 ± 1.4 40.9 ± 2.4(^{*})</td>
</tr>
<tr>
<td>PaCO(_2) kPa</td>
<td>MB</td>
<td>4.7 ± 0.4 4.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>MVB</td>
<td>4.8 ± 0.2 5.4 ± 0.3(^{*})</td>
</tr>
<tr>
<td>pHa</td>
<td>MB</td>
<td>7.38 ± 0.02 7.36 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>MVB</td>
<td>7.38 ± 0.02 7.34 ± 0.02(^{*})</td>
</tr>
<tr>
<td>Lactate (mmol L(^{-1}))</td>
<td>MB</td>
<td>0.54 ± 0.22 0.76 ± 0.47(^{††})</td>
</tr>
<tr>
<td></td>
<td>MVB</td>
<td>0.43 ± 0.19 0.42 ± 0.30(^{*})</td>
</tr>
<tr>
<td>RT (°C)</td>
<td>MB</td>
<td>37.9 ± 0.5 37.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>MVB</td>
<td>37.6 ± 0.2 37.4 ± 0.4</td>
</tr>
</tbody>
</table>
## Results

At 10 minutes after IM administration of the premedication (MB or MVB), significant decreases in HR ($p < 0.001$) and CI ($p < 0.004$), and significant increases in CVP ($p < 0.001$) and SVRI ($p < 0.007$) were detected with both treatments (Fig. 1 & Table 1). Values were significantly lower with MB than with MVB in HR and CI, and significantly larger with MB compared to MVB in CVP, MAP and SVRI ($p < 0.001$). After ketamine, HR ($p < 0.001$) and CI ($p < 0.004$) increased significantly, and SVRI ($p < 0.001$) decreased significantly with both treatments when compared with values at -10 minutes. MAP was significantly lower in MVB than MB after ketamine, and MAP of 57 to 59 mmHg was observed in two dogs 40 and 55 minutes after ketamine ($p < 0.007$). Atipamezole administration decreased SVRI ($p < 0.001$) significantly and increased CI ($p < 0.005$) significantly with both treatments 10 minutes after administration when compared with values at 55 minutes.

Fr decreased significantly from baseline at -10 minutes in MB and MVB (Table 2). Fr stayed significantly lower than baseline throughout the observation period with both treatments ($p < 0.001$). In MVB, PaO$_2$ (range: 69.1–95.9 mmHg, 10.8–13.2 kPa) decreased significantly ($p = 0.046$) and PaCO$_2$ (range: 37.5–43.6 mmHg, 5.0–5.8 kPa) increased significantly ($p = 0.013$) at -10 minutes. Arterial pH and lactate were significantly lower in MVB than MB at -10 minutes ($p = 0.016$ and $p < 0.001$, respectively). Assisted ventilation for 1–16 minutes was needed to treat apnoea in six of the eight dogs in both treatments immediately after ketamine administration. PaCO$_2$ increased significantly at 10 minutes with both treatments when compared with values at -10 minutes ($p < 0.001$). Increase in PaCO$_2$ was significantly greater in MB (range: 51.8–70.1 mmHg, 6.9–9.3 kPa) than in MVB (range: 48.2–59.2 mmHg, 6.4–7.9 kPa) at 10 minutes after ketamine administration ($p = 0.06$).

Composite sedation scores indicated marked sedation after both premedications. There were no significant differences between the treatments in induction scores, jaw tone or intubation scores. Induction score was assessed ideal in all dogs with both treatments and the mouth could be opened without any resistance in six dogs in MB and in seven dogs in MVB. Mild coughing during or immediately after endotracheal intubation was recorded in two dogs in MB and in three dogs in MVB.

Composite sedation score was lower in MVB than MB at 55 and 60 minutes after ketamine administration ($p < 0.001$). Exubation was performed at 52 ± 14 and 62 ± 14 minutes in MVB and MB, respectively, after ketamine administration. Six dogs in MVB and one dog in MB were extubated before administration of atipamezole. After atipamezole was administered at 60 minutes, dogs were alert at 64 ± 11 and 68 ± 4 minutes in MVB and MB, respectively. With MVB, the transition to alertness was fairly easy in five dogs and easy in two dogs, but one dog was restless during early recovery. With MB transition was easy in seven dogs and fairly easy in one dog. Late recovery scores at 120 minutes after ketamine administration did not differ between treatments; six dogs were alert and responsive and two dogs were slightly sedated or slow to react at the last observation time. Nausea, expressed as drooling, swallowing or lack of appetite, was recorded in three dogs in MVB and in two dogs in MB. Defaecation was observed in one dog in MB and tenesmus with mucous faeces in one dog in MVB.

Dexmedetomidine plasma concentration was significantly higher 90 minutes and butorphanol concentration 30 minutes after IM administration at -20 minutes in MVB than MB (Fig. 2). Ketamine plasma concentration was significantly higher 10 minutes after IV administration with MB than MVB. The AUC of ketamine at 10–70 minutes was significantly smaller in MVB (36,700 ± 5200 minute ng mL$^{-1}$) than MB (63,600 ± 14,700 minute ng mL$^{-1}$).

## Discussion

Overall, vatinoxan attenuated the medetomidine-evoked decreases in HR and CI by reducing SVRI but did not completely prevent the early cardiovascular changes. Full pharmacokinetic evaluation during the absorption phase of medetomidine, butorphanol and vatinoxan could not be performed in the present study. However, in previous studies it has been suggested that a probable explanation is that vatinoxan accelerates the absorption and distribution of concomitantly IM administered drugs, by reducing vasoconstriction systematically and at the injection site, while the absorption rate of vatinoxan is slower (Restiutti et al. 2017; Kallio-Kujala et al. 2018). At the same time, the central effects of medetomidine are unaffected by vatinoxan, depressing HR and CO when compared to an alert, conscious state.

SVRI and MAP decreased and CI and HR increased after ketamine administration in both treatments. These results are in agreement with those of a previous study where ketamine administered IV approximately 5 minutes after IV administration of an $\alpha_2$-adrenoceptor agonist, xylazine, induced moderate haemodynamic changes with a decrease in SVR and increases in both HR and CO (Haskins et al. 1986). In the present study, the increase in CI was attributed mainly to an increase in HR because SVI was not increased. The significant decrease in CVP in MVB may have resulted from decreased preload by less central shifting of peripheral venous blood or lower SVRI and higher CI. The present study demonstrated that once medetomidine’s initial vasoconstrictive effects have waned or been prevented by coadministration of vatinoxan, ketamine can be used to treat both the bradycardia and decrease in CO. However, since the ketamine-induced elevations in HR and CI were transient, it seems that sympatholytic effects of medetomidine overrode some of ketamine’s sympathomimetic effects. Hence, repeated or continuous ketamine administration might be needed to achieve more sustained effect.
Bloor et al. (1992) reported that dexmedetomidine decreased plasma noradrenaline and adrenaline concentrations below those that have impact on the cardiovascular system and that after administration of atipamezole, noradrenaline and adrenaline concentrations returned to baseline. Ketamine has direct cardiovascular depressant effects that are usually masked by the ensuing sympathetic stimulation opposing its direct vasodilatory and myocardial depressant effects (Diaz et al. 1976). The positive inotropic effects of ketamine are indirect and contributed to increased sympathetic nervous system activity (White et al. 1982). Medetomidine sympatholytic properties (i.e. decrease in sympathetic outflow from the central nervous system and decreased catecholamine concentrations) may, however, blunt ketamine inotropic effects. Although studies with measurement of plasma catecholamine concentrations would be informative, the results of the current study indicate that vatinoxan did not antagonize the medetomidine-induced inhibition of noradrenaline and adrenaline release; an effect that was reversed by atipamezole. This latter effect indicates that the central cardiovascular depressor effects of medetomidine probably contributed to the decreased HR and CI following initial effects of ketamine.

Hypotension was recorded in two dogs in MVB before atipamezole administration. Overall, MAP remained higher in MB because of increased SVRI. Despite the recorded hypotension, it is probable that tissue perfusion may have been better maintained because CI was significantly higher and SVRI lower in MVB than MB. No differences in RPP were detected between treatments, probably because the differences in MAP were opposed by those in HR. Medetomidine administered alone induces a biphasic blood pressure response with an initial increase in MAP resulting from peripheral vasoconstriction (Schmeling et al. 1991; Bloor et al. 1992; Flacke et al. 1993). Then MAP decreases via a central sympatholytic effect, becoming more apparent as vasoconstriction wanes (Schmeling et al. 1991; Bloor et al. 1992; Flacke et al. 1993). Vatinoxan ameliorates the early hypertensive effect of ζ2-adrenoceptor agonists by blocking vascular ζ2-adrenoceptors (Pagel et al. 1998), and in the absence of the characteristic ζ2-adrenoceptor agonist induced vasoconstriction, the late depressor effects may result in decreased MAP. IV administration of vatinoxan combined with dexmedetomidine at the same dose ratio of 1:25 as in the present study, did not result in significant reductions in MAP in conscious dogs (Honkavaara et al. 2011). Furthermore, no hypotension developed when vatinoxan was combined with medetomidine and butorphanol (Salla et al. 2014). Hence, cardiovascular actions of ketamine probably influenced the outcome in the present study.

It is worth noting, that apart from being endotracheally intubated, the dogs in the present study were not stimulated. In clinical practice, any surgical or other procedural stimuli may also modify cardiovascular function. To summarize, combining drugs with vasodilatory, sympatholytic, negative inotropic or chronotropic effects may exacerbate the decrease in MAP when used together with vatinoxan.

A limitation of the current study was that there were no treatments without ketamine. $f_A$ decreased significantly after premedication in both treatments. Assisted ventilation was required in 75% of the dogs after injection of ketamine and subsequently $\text{PaCO}_2$ increased significantly in both treatments. These effects are similar to a previous study reporting apnoea in 50% of the dogs administered medetomidine (10 $\mu$g kg$^{-1}$) and ketamine (4 mg kg$^{-1}$) (Ko et al. 2001). The selection of a 4 mg kg$^{-1}$ dose of ketamine in the present study was to ensure appropriate conditions for tracheal intubation (Ko et al. 2001) and to document the cardiovascular effects (Pagel et al. 1992), but the dose may have exacerbated the degree of postinduction apnoea. Administration of vatinoxan did not affect the incidence of apnoea after ketamine. When using these premedication combinations with ketamine, preoxygenation is recommended and a method to support ventilation following intubation should be available.

The quality of anaesthesia induction and conditions for endotracheal intubation were not different between the pretreatments, eventhough plasma concentration of ketamine was significantly lower 10 minutes after IV administration and premedication with vatinoxan. This was probably because of vatinoxan-improved haemodynamic function better maintaining the blood flow to liver in MVB than MB. Overall smaller AUC of ketamine manifested clinically as shorter duration of anaesthesia in MVB than MB.

The quality of recovery after atipamezole administration in the present study was less than optimal in some dogs that exhibited nausea and/or defaecation. Ketamine is reported to cause salivation and defaecation in dogs (Haskins et al. 1985; Jacobson & Hartsfield 1993). Atipamezole induces defaecation in dogs by abolishing the inhibitory effect of medetomidine on colonic motility mainly via activation of peripheral ζ2-adrenoceptors (Maugeri et al. 1994). Thus, it should be expected that vatinoxan may increase colonic smooth muscle tone and restore motility. Vomiting and defaecation have been reported in dogs recovering from sevoflurane anaesthesia and vatinoxan administration (Hector et al. 2017). It is probable that the nausea and defaecation observed in approximately 30% of the dogs in the present study was a consequence of multiple drug interactions.

Cardiovascular results after atipamezole administration might have been affected by the gastrointestinal distress that some of the dogs experienced, since the increase in HR and CI was much larger compared to previous study by Turunen et al. (2019). However, the effect might have been exaggerated also because atipamezole is a competitive ζ2-adrenoceptor antagonist, and its dose should be adjusted according to timing of previously administered medetomidine. In the present study atipamezole was administered with a label dose of 100 $\mu$g kg$^{-1}$, although 80 minutes had already passed from premedication. Administration of atipamezole with decreased dose might have reduced gastrointestinal side effects and overstated cardiovascular response.
Conclusions
Vatinoxan improved hemodynamic function in dogs when coadministered with medetomidine and butorphanol as premedication for ketamine anaesthesia, although mild hypotension was observed in 25% of the dogs with the 1 medetomidine-to-25 vatinoxan dose ratio used in this study. Vatinoxan did not influence the clinical quality of anaesthesia induction or intubation, but the duration of anaesthesia was shorter. Atipamezole could be used to hasten the recovery but its dose should be adjusted to improve the quality of recovery.

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Author’s contributions
HT; study design, conducted the study, data analysis, drafted the manuscript; MR study design, conducted the study, reviewed and edited the manuscript; JH study design, reviewed and edited the manuscript; JJ conducted the study, reviewed and edited the manuscript; MS analytical methods, reviewed and edited the manuscript; SM analytical methods, reviewed and edited the manuscript; HH analytical methods, reviewed and edited the manuscript; RB study design, conducted the study, reviewed and edited the manuscript; OV study design, reviewed and edited the manuscript.

Conflict of interest statement
HT is currently employed by Vetcare Oy. Vetcare Oy did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript. The laboratory of MS has been engaged in contract research for Vetcare Oy. Other authors declare no conflicts of interests.
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Original article

Peripherally acting α-adrenoceptor antagonist MK-467 with intramuscular medetomidine and butorphanol in dogs: A prospective, randomised, clinical trial

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A B S T R A C T

The aim of this study was to investigate the clinical usefulness of MK-467 (vatinoxan; L-659'066) in dogs sedated for diagnostic imaging with medetomidine-butorphanol. It was hypothesised that MK-467 would alleviate bradycardia, hasten drug absorption and thus intensify the early-stage sedation. In a prospective, randomised, blinded clinical trial, 56 client-owned dogs received one of two IM treatments: (1) 0.5 mg/kg2 medetomidine + 0.1 mg/kg butorphanol (MB, n = 29); or (2) 0.5 mg/kg medetomidine + 0.1 mg/kg butorphanol + 10 mg/m2 MK-467 (MB-MK, n = 27). Heart rates and visual sedation scores were recorded at intervals. Plasma drug concentrations were determined in venous samples obtained approximately 14 min after injection. Additional sedation (50% of original dose of medetomidine IM) and/or IM atipamezole for reversal were given when needed. The area under the sedation score-time curve for visual analogue scale (AUCVAS) was calculated for the first 30 min after treatment using the trapezoidal method. Repeated ANOVA, Mann–Whitney U test and Fisher’s exact test were used for parametric, non-parametric and dichotomous data. Heart rate was significantly higher from 10 to 40 min with MB-MK than with MB. AUCVAS was significantly higher after MB-MK. More dogs treated with MB-MK required additional sedation after 30 min, but fewer needed atipamezole for reversal compared with MB. Plasma concentrations of both medetomidine and butorphanol were higher after MB-MK. All procedures were successfully completed. MK-467 alleviated the bradycardia, intensified the early stage sedation and shortened its duration in healthy dogs that received IM medetomidine-butorphanol.

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Introduction

The α2-adrenoceptor agonists medetomidine and dexmedetomidine produce reliable sedation and some degree of analgesia, permitting minor procedures to be performed in clinical veterinary practice (Scheinin and MacDonald, 1989; Murrell and Hellebrekers, 2005). Butorphanol is a synthetic opioid that is frequently used with medetomidine to enhance the quality of sedation in dogs (Pyndepop et al., 1996; Kuo and Keegan, 2004). However, all α2-adrenoceptor agonists have adverse effects, mainly related to depression of the cardiovascular system. Specifically, α2-adrenoceptor agonists induce vasoconstriction (Bloor et al., 1992), followed by marked baroreflex-mediated bradycardia (Pyndepop and Verstegen, 1998). The bradycardia is associated with pronounced decreases in cardiac output and oxygen delivery (Bloor et al., 1992; Pyndepop and Verstegen, 1998), an outcome that challenges the usefulness of these drugs.

Although the beneficial effects (i.e. sedation and analgesia) of α2-adrenoceptor agonists are produced at the level of the central nervous system (CNS), activation of peripheral α2-adrenoceptors located within vascular smooth muscle leads to the initial vasoconstriction and related cardiovascular effects (Clough and Hatton, 1981; Horn et al., 1982). In view of this, MK-467 (vatinoxan; L-659'066), a peripherally acting α2-adrenoceptor antagonist (Clineschmidt et al., 1988), has been investigated for its ability to prevent or attenuate the negative impact of dexmedetomidine and medetomidine in dogs (Pagel et al., 1998; Enoori et al., 2008; Honkavaara et al., 2008; Honkavaara et al., 2011).

Only a small proportion of MK-467 crosses the blood–brain barrier into the mammalian CNS (Clineschmidt et al., 1988). Thus,
the pharmacodynamic actions of MK-467 are limited to tissues and organs outside the blood–brain barrier. Several studies have demonstrated that MK-467 is able to prevent cardiovascular depression in dogs (Pagel et al., 1998; Enouri et al., 2008; Honkavaara et al., 2008, 2011) without substantially altering the sedation elicited by medetomidine and medetomidine (Honkavaara et al., 2008; Restitutti et al., 2011, 2017). It has also been shown that concomitant administration of MK-467 attenuates the cardiovascular effects of a medetomidine–butorphanol combination when both are given by intramuscular (IM) injection in the same syringe (Salla et al., 2014). Furthermore, an increase in the absorption rate of medetomidine, when combined with MK-467 for IM administration, has been reported (Restitutti et al., 2017).

To date, studies on MK-467 in dogs have been performed using laboratory beagles under controlled, experimental conditions. Hence, our aim was to investigate the effects of MK-467 on sedation and bradycardia expected after IM administration of medetomidine and butorphanol in healthy dogs of various breeds in a clinical environment. We hypothesised that MK-467, when co-administered IM with medetomidine and butorphanol, would attenuate bradycahdia without impairing the sedative action of this commonly used combination.

Materials and methods

Animals

After receiving approval from the National Animal Experimental Board of Finland (approval number ESAVI-4082/04/10.07.2016, Date of approval 10th October 2016), the study was performed at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, University of Helsinki, Finland. The target population was client-owned dogs that required sedation for non-invasive radiographic imaging. Inclusion criteria were weight >5 kg, age from 3 months to 10 years and American Society of Anesthesiologists (ASA) classification I and II. Exclusion criteria were breed-related contraindication for deep sedation (e.g. brachycephalic syndrome), systemic disease or medications affecting the CNS. Informed consent was obtained from the owners. Most of the dogs enrolled in the study were scheduled for radiographic imaging required by the Finnish Kennel Club Health Programme for the screening of canine genetic diseases and defects.

Experimental design

In a randomised, complete, block design, animals were assigned to receive one of two treatments: (1) 0.5 mg/kg medetomidine (HCl 0.1 mg/kg butorphanol tartrate (MB); or (2) 0.5 mg/kg medetomidine HCl 0.1 mg/kg butorphanol tartrate 10 mg/ml MK-467 HCl (MB-MK). The body surface area was calculated using the following formula: body surface area (m$^2$) = 10.4 x (weight in kg)$^{0.625}$ x 10.2 (White and Kearney, 2014; Pyepeldon and Jones, 2015). The dose of medetomidine HCl (0.5 mg/kg) was equivalent to a dose of 29.5 μg/kg for a 5 kg dog and 11.7 μg/kg for an 80 kg dog. A solution containing 0.5 mg/ml medetomidine HCl for the MB treatment and a solution containing 0.5 mg/ml medetomidine HCl and 10 mg/ml MK-467 HCl (Vetcare Oy; Recipharm) was used for the MB-MK treatment. Butorphanol (Torquopur 10 mg/ml, Richter Pharma AG) was drawn up separately and mixed with the solution containing medetomidine before administration. The end volume of the injectable solution in both treatments was 0.03 to 0.07 ml/kg (0.57 ml for a 10 kg dog), depending on the weight. Randomisation (Microsoft Office Excel) into treatment groups was performed in blocks for breed (Retrievers, Shepherds, Spitzs and the group 'Other breeds') and weight (small dogs 5–10 kg, medium size dogs 11–30 kg and large dogs >31 kg) to ensure relatively homogeneous populations between treatments. Treatments were administered IM into the gluteal muscles.

Ten minutes after drug injection, a catheter was inserted in a cephalic vein aseptically and blood was drawn into tubes containing ethylene diamine tetra-acetic acid (EDTA) for plasma drug concentration analyses and complete blood counts, and into a serum tube for basic serum chemistry. Blood samples obtained later than 20 min after drug injection were excluded from the plasma drug concentration data. The total volume harvested was <10 ml, representing no more than 3% of the total blood volume of a 5 kg dog. Plasma was separated by centrifugation at 2300 g for 10 min within 30 min after collection and stored at −20 °C until analysis for medetomidine, butorphanol and MK-467 concentrations. Oxygen was supplemented with a loose mask at 2–4 l/min according to the dog’s size. Prior to treatment administration, heart rate (HR) was assessed by auscultation, respiratory rate was assessed by observation of thoracic movements, colour of the mucous membranes was assessed by direct observation and the level of sedation was scored. The evaluations were repeated at 5 min after treatment and thereafter at 10 min intervals. Rectal temperatures were measured prior to treatment administration and at every 30 min. Dogs were passively insulated with blankets; if the body temperature decreased <37 °C, they were warmed actively by a convective temperature management system (Bair-Hugger, 3M).

The primary investigator who assessed the sedation was blinded to the treatment. A second investigator administered the treatments and recorded the HRs and other clinical variables. Sedation was determined using a visual analogue scale (VAS) from 0 to 10, where 0 represents no sedation and 10 represents the animal in lateral recumbency, unresponsive to a loud hand clap. The ‘area under the sedation score time’ curve for VAS was calculated for the first 30 min after treatment (AUCVAS30) using the trapezoidal method. ‘Head down time’ was recorded and the time when the dog was able to become responsive to a loud hand clap. If the level of sedation was inadequate for performing the radiographic imaging, as judged by the blinded investigator, 50% of the initial medetomidine dose (without butorphanol and MK-467) was administered IM. Treatment failure was defined as insufficient sedation after the additional medetomidine.

The duration of required sedation was noted as the time from the IM injection until the radiographic imaging was concluded. After successful completion of the procedure, the blinded investigator administered 0.625 mg/ml atipamezole IM (37 μg/kg for a 5 kg dog; Alzane 5 mg/ml, Laboratorios SYVA S. A.U.) if the dog was not able to stand or walk. If the dog was not able to stand on its own after the first dose, the same dose was re-administered 10 min later. The evaluations were continued as described above until the animal was standing. The owner was interviewed by telephone the following day regarding the overall behaviour of the dog: (1) was the dog more lethargic than normal in the evening after sedation; (2) was there anything else out of the ordinary; and (3) were there any changes in appetite, faeces or behaviour? Owners were blinded to the treatment until the end of the telephone call.

Analytical methods

Concentrations of medetomidine, butorphanol and MK-467 in plasma were measured using liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS; Waters Acuity UPLC + Waters TQ–3 triple quadrupole MS). After precipitation of 100 μl plasma samples in a 96-well precipitation plate (Waters Sirocco Waters) with 200 μL acetonitrile containing 20 ng/ml chlorpromazine as an internal standard, the plasma supernatants were transferred to new 96-well plates and a 50 μl aliquot was transferred to another 96-well plate and diluted 1:10 in 20X aqueous acetonitrile prior to analysis. Diluted and undiluted samples were analyzed. Reference standards were prepared by ‘spiking’ blank dog plasma with the analytes, producing final concentrations in the range 0.02–20,000 ng/ml. The linear calibration ranges were fitted as 0.2–500 ng/ml for undiluted butorphanol, 0.2–2000 ng/ml for diluted medetomidine and 0.5–2000 ng/ml for undiluted MK-467. The quality control samples were within 94–116% of the nominal concentrations.

Statistical analysis

A power analysis, based on mean HRs of 40 (MB) and 60 (MB-MK) beats per min (bpm), with a standard deviation of 15 bpm, suggested that 20 dogs per group was required with a power of 80% and a level of 0.05. The Shapiro–Wilks test was used to evaluate the normality of data distributions. Heart and respiratory rates were evaluated by analysis of variance (ANOVA; general linear model), where the result was a within-subject factor and treatment was a between-subject factor. A t-test with the Holm–Bonferroni post-hoc correction was used for comparisons between treatments and against baseline. The independent samples t test was performed on plasma drug concentrations and time for ‘head down’. Areas under the curve (AUCVAS30) were compared using the Mann–Whitney U test. VAS and pale mucous membranes were compared between the two treatments at each time point using the Mann–Whitney U test and against baseline within each treatment using the related samples Friedman’s two-way ANOVA by ranks. The Holm–Bonferroni correction was used with both comparisons. The need for additional medetomidine or atipamezole, presence of loose faeces, lethargy and decreased appetite were compared using the two-sided Fischer’s exact test. Analyses were computed using SPSS Statistics version 24.0 for Windows (IBM). P values <0.05 were considered to be statistically significant.

Results

The MB group contained 29 dogs and the MB-MK group contained 27 dogs (Table 1). None of the dogs had pertinent alterations in haematology or serum chemistry considered to be consequential to the investigated outcomes. All the procedures were completed successfully and no treatment failures occurred in either group. Side effects, considered to be minor, are reported in Table 2.
Table 1
Characteristics of 56 dogs undergoing sedation with either MB (n = 29) or MB-MK (n = 27) for radiography.

<table>
<thead>
<tr>
<th>MB</th>
<th>n</th>
<th>MB-MK</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)*</td>
<td>18.6 (6.3–42.0)</td>
<td>24.6 (8.8–71.0)</td>
<td></td>
</tr>
<tr>
<td>Age (years)*</td>
<td>2.3 (1.1–8.8)</td>
<td>2.9 (1.1–9.4)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>12</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>17</td>
<td>Female</td>
</tr>
<tr>
<td>Breed</td>
<td>German shepherd</td>
<td>4</td>
<td>German shepherd</td>
</tr>
<tr>
<td></td>
<td>Border collie</td>
<td>3</td>
<td>Flat coat retriever</td>
</tr>
<tr>
<td></td>
<td>Giant schnauzer</td>
<td>2</td>
<td>Lapphund</td>
</tr>
<tr>
<td></td>
<td>Golden retriever</td>
<td>2</td>
<td>Lapponian herder</td>
</tr>
<tr>
<td></td>
<td>Lapponian herder</td>
<td>2</td>
<td>Whippet</td>
</tr>
<tr>
<td></td>
<td>Mudi</td>
<td>2</td>
<td>American Staffordshire bull terrier</td>
</tr>
<tr>
<td>Mixed breed</td>
<td>2</td>
<td>Australian shepherd</td>
<td>1</td>
</tr>
<tr>
<td>Breed</td>
<td>Bohemian shepherd</td>
<td>1</td>
<td>Bassett hound</td>
</tr>
<tr>
<td></td>
<td>Cairn terrier</td>
<td>1</td>
<td>Bedlington terrier</td>
</tr>
<tr>
<td></td>
<td>Cirneco delletta</td>
<td>1</td>
<td>Border collie</td>
</tr>
<tr>
<td></td>
<td>Cocker spaniel</td>
<td>1</td>
<td>Cairn terrier</td>
</tr>
<tr>
<td></td>
<td>Flat coated retriever</td>
<td>1</td>
<td>Cocker spaniel</td>
</tr>
<tr>
<td></td>
<td>Miniature</td>
<td>1</td>
<td>Entlebucher sennenhund</td>
</tr>
<tr>
<td></td>
<td>Schnauzer</td>
<td>1</td>
<td>Whippet</td>
</tr>
<tr>
<td></td>
<td>Podenco</td>
<td>1</td>
<td>Giant schnauzer</td>
</tr>
<tr>
<td></td>
<td>Schweizer laufhund</td>
<td>1</td>
<td>Golden retriever</td>
</tr>
<tr>
<td></td>
<td>Whippet</td>
<td>1</td>
<td>Mixed breed</td>
</tr>
<tr>
<td></td>
<td>Norwegian elkhound</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

MB. 0.5 mg/m² medetomidine + 0.1 mg/kg butorphanol; MB-MK. 0.5 mg/m² medetomidine + 10 mg/m² MK-467 + 0.1 mg/kg butorphanol.

The group size of a breed for each treatment is expressed as the number of individuals of that breed.

* Median and range in curved brackets. No significant differences were detected between groups in weight, age or sex.

HRs were reduced from baseline after both treatments; however, HR was significantly higher in the MB-MK group than in the MB group (Fig. 1). HRs were reported until 40 min; at that time point, two dogs were missing from each group, representing the end of the procedure for one dog in each group and the requirement for additional sedation in one dog in each group. The lowest HRs detected after additional treatment with medetomidine were 28 bpm in the MB group (n = 1) and 34 bpm in the MB-MK group (n = 7). One dog in the MB group had an increased HR after drug administration (up to 148 bpm); this was considered to be an outlier, and the HR data for this dog were excluded from Fig. 1 and from the statistical analyses.

Discussion

The findings of this study, using a population of healthy dogs in a clinical setting, indicate that administration of MK-467 IM concomitantly with medetomidine and butorphanol alleviates medetomidine-related bradycardia. Profound bradycardia (approximately 40 bpm) was detected in dogs treated with medetomidine and butorphanol (MB group). The HR was also low in dogs treated with medetomidine, butorphanol and MK-467 (MB-MK group; mean HR approximately 60 bpm). However, similar or even lower HRs have been detected in untreated, healthy sleeping dogs; nightly HRs ~60–70 bpm have been reported in beagle dogs (Nolan et al., 2004; Honkavaara et al., 2008).

Moreover, in healthy, adult, pet dogs, minimum HRs of 17–46 bpm have been detected on ambulatory electrocardiography (Hall et al., 1991) and, in a more recent study, the mean of the minimum HRs was also <50 bpm (Cruz Aleixo et al., 2017). In our study, HR was significantly lower than baseline in the MB-MK group, which suggests that MK-467 did not override the central component of the bradycardic action of α2-agonists (Savola, 1986), although it was presumed to have been able to alleviate the baroreflex-mediated bradycardia caused by peripheral α2-adrenoceptor activation.

The present results should not be over-interpreted, since we did not show that cardiac output or oxygen delivery were improved by the addition of MK-467. However, in experimental canine studies,

Table 2
Results for 56 dogs undergoing sedation with either MB (n = 29) or MB-MK (n = 27) for radiography.

<table>
<thead>
<tr>
<th>MB</th>
<th>MB-MK</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head down (s)*</td>
<td>297.5 ± 74.3</td>
<td>241.4 ± 77.8</td>
</tr>
<tr>
<td>Pale mucous membranesb</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Additional medetomidine (min)*</td>
<td>1 (30)</td>
<td>7 (33 ± 10)</td>
</tr>
<tr>
<td>Atipamezole administrationb</td>
<td>26</td>
<td>12</td>
</tr>
<tr>
<td>Duration of required sedation (min)*</td>
<td>66 ± 14.5 (30–94)</td>
<td>66 ± 19 (42–111)</td>
</tr>
<tr>
<td>Lethargyb</td>
<td>26</td>
<td>17</td>
</tr>
<tr>
<td>Loose faecesb</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Decrease in appetitеб</td>
<td>23</td>
<td>25</td>
</tr>
<tr>
<td>Blood sample analysedb</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Blood sampling time (min)*d</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Medetomidine (ng/mL)*</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Butorphanol (ng/mL)*</td>
<td>607 ± 204</td>
<td></td>
</tr>
</tbody>
</table>

MB. 0.5 mg/m² medetomidine + 0.1 mg/kg butorphanol; MB-MK. 0.5 mg/m² medetomidine + 10 mg/m² MK-467 + 0.1 mg/kg butorphanol.

Lethargy, loose faeces and decrease in appetite reported by owners.

One dog in both groups received additional medetomidine and one dog in both groups received additional atipamezole.

* Mean ± standard deviation.

b Number of dogs.

c Range in curved brackets.

d Median.

VAS and AUCVAS are shown in Table 3. In the MB-MK group, more dogs required additional sedation after 30 min than in the MB group. In addition, fewer dogs in the MB-MK group required administration of atipamezole (Table 2). Respiratory rates decreased after both treatments, with no significant difference between groups. The lowest respiratory rates were detected at 20–30 min, with medians of 8 and 10 breaths per min in the MB-MK and MB groups, respectively. None of the dogs had a rectal temperature below 37 °C. Due to technical problems there were many missing blood pressure readings (data not analysed nor shown).

Plasma concentrations of both medetomidine and butorphanol were significantly higher in the MB-MK group in samples collected 11–18 min after drug injection (Table 2). ‘Head down’ time, mucous membrane colour, duration of required sedation, lethargy, loose faeces, decrease in appetite, number of blood samples analysed and sampling time are shown in Table 2.
Improvement in the alleviation of bradycardia by MK-467 has been associated with improvement of cardiac output (Honkavaara et al., 2011; Salla et al., 2014). The lack of blood pressure data in our study is a major limitation; blood pressure was monitored non-invasively and blood pressure measurements could not be carried out systematically at the prescribed same time points, nor using the same artery, because the clinical priority was to avoid excessive interference with the radiological exams. Therefore, we did not consider the data to be reliable and further studies using clinical cases are needed.

The onset of sedation appeared to be faster in the MB-MK group and, initially, was deeper than in the MB group. The ‘head-down’ time was significantly shorter in the MB-MK group and we detected a deeper overall sedation during the first 30 min. Subsequently, dogs in the MB-MK group were more alert than dogs in the MB group, since more additional medetomidine and less atipamezole were required. Thus, the use of MK-467 can be considered advantageous, especially if short, intense, sedation is required. If additional medetomidine was needed, atipamezole alone was given to dogs in both groups, since no studies have been performed on the effects of repeated doses of MK-467 in sedated dogs. Since the need for additional sedation arose after a mean of 52 min in dogs in the MB-MK group, the duration and magnitude of the sedative effect of medetomidine and butorphanol combined with MK-467 could provide sufficient sedation to complete a minor non-invasive procedure. The quality and level of sedation induced by medetomidine in our study probably were improved by butorphanol, independent of MK-467, in accordance with that reported previously (Pypendop et al., 1996; Ko et al., 2000).

Conversely, the effects of MK-467 on the plasma concentration profile of medetomidine and butorphanol apparently also affected the depth of sedation.

The interactions between α2-adrenoceptors and their antagonists start at the site of the extravascular injection; MK-467 enhances the IM absorption of medetomidine, probably by preventing local vasoconstriction caused by medetomidine (Restitutti et al., 2017) and it also appears to affect the absorption of other co-administered sedatives (Kallio-Kujala et al., 2017). In our study, plasma medetomidine and butorphanol concentrations were significantly higher in the MB-MK group than in the MB group. The plasma sample was collected approximately 14 min after the injection, when we expected to detect a clear difference between the treatments based on our previous findings (Kallio-Kujala et al., 2017; Restitutti et al., 2017). Furthermore, Restitutti et al. (2017) reported that the time-concentration curves of dexmedetomidine intersected at approximately 30 min; the plasma concentration of medetomidine was higher in the presence of MK-467 before the 30 min time point, whereas later it was higher in dogs that had received medetomidine alone IM. These effects of MK-467 on the plasma concentration profiles of medetomidine and butorphanol probably explain both the deeper initial sedation observed in the MB-MK group and the later lighter plane of sedation in the MB-MK group when compared with the MB group.

The most frequent side effects after sedation in both groups were lethargy and loose faeces during the evening after the examination. All dogs that had loose faeces had received one or both of the α2-adrenoceptor antagonists MK-467 and/or atipamezole. Atipamezole restores intestinal motility after α2-adrenoceptor agonist-induced sedation and induces defaecation in dogs (Maugeri et al., 1994). Moreover, frequent defaecation after administration of MK-467 has been reported in horses (DeVries et al., 2016). Therefore instead of giving atipamezole in one single dose, we administered two equal (0.625 mg/m2) smaller doses if needed, to reduce the risk of intestinal hypermotility, especially in the presence of MK-467, but still to have the desired effect of reversing the sedation. Honkavaara et al. (2008) administered atipamezole 50 μg/kg (1.25 mg/m2 for a 16 kg dog) to reverse sedation induced by IV dexmedetomidine and MK-467.

Conclusions

MK-467 alleviates the bradycardia induced by medetomidine in dogs in a clinical setting, and provides reliable sedation for short

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**Table 3**

Visual analogue sedation score (0–100) and the area under the sedation score time curve (AUC) for visual analogue scale (VAS) for the first 30 min \( (\text{AUC}_{\text{VAS}30}) \).

<table>
<thead>
<tr>
<th>Time point (min)</th>
<th>MB</th>
<th>MB-MK</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0–0) ( n=29 )</td>
<td>0 (0–0) ( n=27 )</td>
</tr>
<tr>
<td>5</td>
<td>58 (9–86) ( n=28 )</td>
<td>64* (21–100) ( n=27 )</td>
</tr>
<tr>
<td>10</td>
<td>96 (31–100) ( n=27 )</td>
<td>100 (51–100) ( n=27 )</td>
</tr>
<tr>
<td>20</td>
<td>100 (82–100) ( n=28 )</td>
<td>100 (78–100) ( n=27 )</td>
</tr>
<tr>
<td>30</td>
<td>100 (83–100) ( n=28 )</td>
<td>100 (71–100) ( n=27 )</td>
</tr>
<tr>
<td>40</td>
<td>100 (90–100) ( n=26 )</td>
<td>100 (14–100) ( n=25 )</td>
</tr>
<tr>
<td>( \text{AUC}_{\text{VAS}30} )</td>
<td>2445 (1855–2585)</td>
<td>2555 (1868–2750)</td>
</tr>
</tbody>
</table>

MB, 0.5 mg/m2 medetomidine + 0.1 mg/kg butorphanol; MB-MK, 0.5 mg/m2 medetomidine + 10 mg/m2 MK-467 + 0.1 mg/kg butorphanol.

Data are reported as median (range).

* Significant difference from baseline.

† Significant difference between treatments.

Fig. 1. Heart rates until 40 min after injection for dogs that did not receive additional atipamezole or atipamezole. *Significant difference between treatments.
term clinical procedures, such as diagnostic imaging, when it is combined with IM medetomidine and butorphanol in healthy dogs. In addition, MK-467 increases the early stage plasma concentration of both medetomidine and butorphanol when administrated IM in the same syringe and results in deeper initial sedation with shorter duration.

Conflict of interest statement

None of the authors of this paper have a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

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References
