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Cardiopulmonary, Sedative, and Drug Disposition Effects of Vatinoxan in Sheep Receiving Dexmedetomidine, Medetomidine, Ketamine and Atipamezole

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ACADEMIC DISSERTATION

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To my family

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ABSTRACT

The impact of the peripherally selective α_2-adrenoceptor antagonist, vatinoxan, on selected pharmacodynamic and pharmacokinetic properties of two selective α_2-adrenoceptor agonists, medetomidine and dexmedetomidine, were investigated in sheep. Moreover, certain interactions between vatinoxan and atipamezole, a specific α_2-adrenoceptor antagonist, were evaluated.

The initial objective of this study was to identify a dose of vatinoxan that would best mitigate the undesirable cardiopulmonary changes produced by intramuscular (IM) medetomidine-ketamine in sheep. Specifically, three doses of vatinoxan (150, 300 and 600 µg/kg) or saline were combined in the same syringe with medetomidine (30 µg/kg) and ketamine (1 mg/kg) and given IM. Systemic hemodynamics, arterial blood gas tensions, clinical sedation and plasma drug concentrations were compared, both before and after reversal with IM atipamezole (150 µg/kg). The middle dose of vatinoxan (300 µg/kg), which appeared to be optimal among the other doses, was then added to medetomidine (30 µg/kg) and co-administered IM, followed by atipamezole for reversal. Last, the influence of intravenous pre-treatment with vatinoxan on dexmedetomidine-induced cardiopulmonary alterations was investigated in sevoflurane-anesthetized sheep.

Following concomitant IM administration, vatinoxan dose-dependently attenuated some of medetomidine's cardiopulmonary side effects. Vatinoxan did not significantly affect the level of sedation or the plasma concentrations of drugs when ketamine was included in the same syringe. Conversely, vatinoxan significantly increased the plasma concentrations of medetomidine, and accelerated the onset and intensified the degree of sedation when compared with the agonist alone. Moreover, recoveries after atipamezole-reversal were more complete in the presence of vatinoxan. No deleterious effects were noted between vatinoxan and atipamezole. Pre-treatment with vatinoxan prevented all dexmedetomidine-induced pulmonary alterations in sheep anesthetized with sevoflurane.

In conclusion, vatinoxan alleviated or prevented the unwanted cardiopulmonary effects of (dex-) medetomidine by blocking the peripheral α_2-adrenoceptors. Presumably, when co-administered IM in the same syringe, vatinoxan accelerated the absorption of medetomidine and increased its concentration in blood, which resulted in a faster and more intense sedation than when the agonist was used alone. Vatinoxan also decreased later exposure to dexmedetomidine, which appeared to improve atipamezole's efficacy to reverse both the central and peripheral effect of the agonist.

LIST OF PUBLICATIONS

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

Study I
Adam, M., Raekallio, M.R., Salla, K.M., Honkavaara, J.M., Männikkö, S., Scheinin, M., Kajula, M., Mölsä, S. & Vainio, O.M. (2018) Effects of the peripherally acting α_2-adrenoceptor antagonist MK-467 on cardiopulmonary function in sheep sedated by intramuscular administration of medetomidine and ketamine and reversed by intramuscular administration of atipamezole. *American Journal of Veterinary Research*, 79, 921–932.

Study II
Adam, M., Raekallio, M.R., Keskitalo, T., Honkavaara, J.M., Scheinin, M., Kajula, M., Mölsä, S. & Vainio, O.M. (2018) The impact of MK-467 on plasma drug concentrations, sedation and cardiopulmonary changes in sheep treated with intramuscular medetomidine and atipamezole for reversal. *Journal of Veterinary Pharmacology and Therapeutics*, 41, 447–456.

Study III
Adam, M., Raekallio, M.R. & Vainio, O.M. (2018) Sedative effect of intramuscular medetomidine with and without vatinoxan (MK-467), and its reversal with atipamezole in sheep. *Veterinary Anaesthesia and Analgesia*, 45, 788–793.

Study IV
Adam, M., Huuskonen, V., Raekallio, M.R., Casoni, D., Mykkänen, A.K., Lappalainen, A.K., Kajula, M., Kallio-Kujala, I.J. & Vainio, O.M. (2018) Cardiopulmonary effects of vatinoxan in sevoflurane-anaesthetised sheep receiving dexmedetomidine. *Veterinary Journal*, 238, 63–69.

ABBREVIATIONS

AUC	Area under the plasma concentration-time curve
BAL	Bronchoalveolar lavage
CaO_2	Arterial oxygen content
C_{dyn}	Dynamic compliance
CI	Cardiac index
CL	Clearance
C_{max}	Maximum plasma drug concentration
CNS	Central nervous system
CO	Cardiac output
CT	Computed tomography
CVP	Central venous pressure
DAP	Diastolic arterial blood pressure
DPAP	Diastolic pulmonary arterial blood pressure
DO_2	Oxygen delivery
$ETCO_2$	End-tidal partial pressure of carbon dioxide
FiO_2	Fraction of inspired oxygen
Hb	Hemoglobin concentration
HR	Heart rate
IBP	Invasive blood pressure
IM	Intramuscular
IPPV	Intermittent positive pressure ventilation
IV	Intravenous
LC-MS/MS	Liquid chromatography & tandem mass spectrometry
LVRPP	Left ventricle rate pressure product
LVW	Left ventricle work
MAP	Mean arterial blood pressure
MPAP	Mean pulmonary arterial blood pressure
M_V	Minute ventilation
$PaCO_2$	Partial pressure of arterial carbon dioxide
PaO_2	Partial pressure of arterial oxygen
$P_{(A-a)}O_2$	Alveolar to arterial difference in partial pressure of oxygen
PEEP	Positive end-expiratory pressure
PIP	Peak inspiratory pressure
$PvCO_2$	Partial pressure of mixed venous carbon dioxide
PvO_2	Partial pressure of mixed venous oxygen
P_{50}	PaO_2 at which hemoglobin is half saturated
ROI	Region of interest
RR, f_R	Respiratory rate
SaO_2	Arterial hemoglobin oxygen saturation percentage
SAP	Systolic arterial blood pressure
SD	Standard deviation
SPAP	Systolic pulmonary arterial blood pressure
SV	Stroke volume
SVR	Systemic vascular resistance
$T_{½}$	Elimination Half-life
T_{max}	Time to reach maximum plasma concentration
V_T	Tidal volume
V_Z	Apparent volume of distribution

1 INTRODUCTION

Alpha$_2$-adrenoceptor agonists are extensively used in veterinary clinical practice for their sedative, anxiolytic, antinociceptive and anesthetic-sparing properties. In ruminants, particularly sheep, their use is associated with cardiopulmonary adverse effects, most notably: arterial hypoxemia, vasoconstriction, and decreases in heart rate, cardiac output and oxygen delivery (Aziz & Carlyle, 1978; Bryant et al., 1996; Celly et al., 1997a; Kästner et al., 2005; Raekallio et al., 2010). The extent of these effects depends mainly on the dose of the agonist and its route of administration. Racemic medetomidine and its active enantiomer, dexmedetomidine, are the most potent and selective α_2-adrenoceptor agonists currently available for veterinary use. In ruminants, medetomidine is widely used either alone or in combination with e.g. ketamine to produce reliable sedation or anesthesia for diagnostic and surgical procedures. The desired sedative effects of α_2-adrenoceptor agonists are mediated via α_2-adrenoceptors within the central nervous system (CNS) (Doze et al., 1989; Maze & Fujinaga, 2000), whereas α_2-adrenoceptors located in the vasculature mediate the initial hemodynamic consequences (Docherty & McGrath, 1980; Kamibayashi & Maze, 2000).

Vatinoxan, previously known as L-659,066 and MK-467, is a peripherally selective α_2-adrenoceptor antagonist that penetrates poorly into the mammalian CNS (Clineschmidt et al., 1988). Over the past decade, various combinations of vatinoxan and α_2-adrenoceptor agonists have been evaluated in several animal species (Bryant et al., 1998; Enouri et al., 2008; Honkavaara et al., 2008; 2011; 2017a, b; Rolfe et al., 2012; Vainionpää et al., 2013; Kaartinen et al., 2014 Salla et al., 2014b; de Vries et al., 2016). The outcomes from these studies are promising as vatinoxan has repeatedly attenuated the peripherally-mediated hemodynamic effects with no clinically relevant effect on the sedative efficacy of the used agonists (Honkavaara et al., 2008; Restitutti et al., 2011; Tapio et al., 2018). The cardiovascular effects of vatinoxan have previously been studied after IV co-administration with dexmedetomidine in sheep (Raekallio et al., 2010). Therefore, the primary aim of this thesis was to further elucidate this interaction in sheep following concurrent IM administration with and without ketamine, focusing primarily on the cardiopulmonary function and sedation. It was hypothesized that medetomidine-induced adverse cardiopulmonary effects would be attenuated by vatinoxan while the sedation would not be negatively impacted. Additionally, the effects of pre-treatment with vatinoxan on dexmedetomidine-induced cardiopulmonary alteration were investigated in sevoflurane-anesthetized sheep. It was postulated that pre-administration of vatinoxan would prevent the increase in airway resistance and pulmonary edema formation, thus attenuating the decrease in arterial partial pressure of oxygen following dexmedetomidine administration.

Atipamezole is a potent, specific antagonist of both centrally and peripherally located α_2-adrenoceptors. It is frequently used to reverse the sedation and cardiopulmonary effects induced by e.g. medetomidine in various animal species (Vainio & Vähä-Vahe, 1990; Raekallio et al., 1991; Arnemo & Søli, 1993b; 1995; Ko & McGrath, 1995; Ranheim et al., 1998; 1999; 2000b; Granholm et al., 2007; Rioja et al., 2008). However, no reports have yet been published on the interaction between atipamezole and vatinoxan in sheep sedated with α_2-adrenoceptor agonists. Thus, it was hypothesized that both antagonists would improve the cardiopulmonary performance in sheep sedated with medetomidine or a combination of medetomidine and ketamine. Additionally, it was postulated that the presence of vatinoxan would decrease the plasma concentration of medetomidine, which could hasten the recovery after atipamezole administration and prevent any resedation.

2 REVIEW OF LITERATURE

2.1 Alpha$_2$-adrenoceptors: Classification, localization, and function

Adrenergic receptors (adrenoceptors) are membrane-bound receptors that mediate various physiological effects via adrenaline, noradrenaline, and their analogues. Although adrenoceptors are structurally related, they have been classified into two subtypes (α and β) based on their relative responsiveness to natural and synthetic amines (Ahlquist, 1948). Later, the α-adrenoceptors were subdivided into α_1 and α_2 classes according their anatomical location, pre- or post-synaptic respectively (Langer, 1974). Alternatively, Berthelsen & Pettinger (1977) proposed a functional classification, where α_1-adrenoceptors mediate excitatory responses and α_2-adrenoceptors mediate inhibitory effects. Presently, the pharmacological classification of α-adrenoceptors, based on the affinity of each receptor for various agonists and antagonists, is widely accepted (Ruffolo et al., 1991). The α_2-adrenoceptors are further subdivided into three subtypes α_{2A}, α_{2B}, and α_{2C} (Lorenz et al., 1990; Blaxall et al., 1991; Bylund, 1992; Bylund et al., 1994; Zhong & Minneman, 1999). A fourth subtype, α_{2D}, was identified in rat submaxillary (Michel et al., 1989) and bovine pineal glands (Simonneaux et al., 1991), albeit now considered to be a species homologue of human α_{2A} subtype (Lanier et al., 1991; Blaxall et al., 1993). On the other hand, α_1-adrenoceptors are subdivided into α_{1A}, α_{1B}, and α_{1D} (Docherty, 2010). A fourth type, named α_{1L}, has been identified based on its low affinity for prazosin and other α_1-antagonists (Flavahan & Vanhoutte, 1986; Hieble, 2007). For β-adrenoceptors, Lands et al. (1967) initially subdivided them into β_1 and β_2 subtypes. Later, a third subtype, β_3, was identified when its encoding gene was isolated by Emorine et al. (1989), and a fourth type has been also been proposed by Hieble (2007).

Pre-synaptic alpha$_2$-adrenoceptors are G-protein coupled and their stimulation inhibit adenylyl cyclase activity through the G_i protein, leading to a decrease in the concentration of cyclic adenosine 3', 5'-monophospate (cAMP) within the cell, thus decreasing the phosphorylation of regulatory proteins (Pichot et al., 2012). Efflux of potassium ions through calcium-activated channels prevents calcium entry into the nerve terminal resulting in an inhibitory effect on secretion of neurotransmitters into the synaptic cleft (Hayashi & Maze, 1993; Piascik et al., 1996) and reduces activity of noradrenergic pathways (Carollo et al., 2008). However, α_2-adrenoceptors are ubiquitously distributed within the mammalian tissues and organs, both pre, post- and extra-synaptically. With α_{2A} being densely expressed within the *locus coeruleus* (Scheinin et al., 1994; MacDonald & Scheinin, 1995), it is the predominant subtype in dog and rat brainstems (Schwartz et al., 1999). In sheep brainstems, α_{2D}, a homologue of α_{2A}, is the predominant subtype (Schwartz & Clark, 1998). Alpha$_{2A}$ mediates sedation and antinociception (Hunter et al., 1997; Lakhalani et al., 1997), anesthetic sparing effects (Lakhalani et al., 1997; Kable et al., 2000), temperature homeostasis (Hunter et al., 1997; Kable et al., 2000), spinal analgesia (Stone et al., 1997), and hyperglycemia (Fagerholm et al., 2004). The α_{2B} receptors are primarily located in peripheral tissues, particularly in vascular smooth muscle fiber, thus mediating the vasoconstrictive response to agonist drugs (Docherty & McGrath, 1980; Link et al., 1996; Makaritsis et al., 1999; Kamibayashi & Maze, 2000; Paris et al., 2003). The peripherally located α_{2C} subtype receptors contribute to vasoconstriction of small arteries (Chotani et al., 2004), whereas the centrally located ones are involved in the hypothermic response to α_2-adrenoceptor agonists and regulate dopamine-mediated effects in the brain (Sallinen et al., 1997; Björklund et al., 1999; Scheinin et al., 2001). On the other hand, postsynaptic α_1-adrenoceptors are

widely distributed in vascular smooth muscle, α_{1A} being the predominant receptor mediating the vasoconstriction in many arteries such as mammary, mesenteric, splenic, hepatic, omental, renal, pulmonary, and the epicardial coronary ones (Rudner et al., 1999). It is also present in veins such as the caval, saphenous, and pulmonary veins (Michelotti et al., 2000). The α_{1B} receptor subtype is the most abundant subtype in the heart, but is present at a low density (Brodde & Michel, 1999), whereas the α_{1D} receptor subtype is the predominant receptor causing vasoconstriction in the largest arteries (Piascik et al., 1995; 1997) such as the aorta (Deng et al., 1996).

2.1.1 Cardiovascular regulation via α-adrenoceptors

Alpha-adrenoceptors play a major role in regulation of vasomotor tone (Piascik et al., 1996). *In vitro* studies demonstrated that α_1-adrenoceptors mediate vasoconstriction in both arterial and venous tissue preparations (Guimaraes & Moura, 2001; Docherty, 2010). Alpha$_{2B}$-receptors, on the other hand, are the main subtypes mediating the arterial vasoconstriction (Link et al., 1996; Makaritsis et al., 1999). However, the peripheral α_{2A} and α_{2C} receptors are also involved in vasoconstriction of distal arteries (Guimaraes & Moura, 2001). The induced peripheral vasoconstriction increases the systemic vascular resistance (SVR) and evokes the baroreceptor reflex arch, which results in bradycardia as demonstrated in autonomically intact dogs (Bloor et al., 1992; Flacke et al., 1993). Similar outcomes have been reported after selective α_1-adrenoceptor agonism by e.g. phenylephrine (Morimatsu et al., 2012). In autonomically denervated dogs the increase in afterload, without bradycardia, was responsible for the decreases in cardiac index (CI) (Flacke et al., 1990). Furthermore, the vasoconstriction of coronary arteries due to activation of α_2-adrenoceptors may induce myocardial dysfunction as a result of decreased oxygen delivery (Flacke et al., 1993), although this may at least partially be prevented by a decrease in myocardial oxygen demand (Willigers et al., 2006). Interestingly, despite their absence in both myocardium and sinoatrial nodes, α_2-adrenoceptor agonists produced indirect myocardial depressant effects in isolated heart preparations (Flacke et al., 1992; Hongo et al., 2016). However, postsynaptic β_1-receptors are the predominant adrenergic receptors within the myocardium, in the non-failing human heart representing approximately 80% of the expressed β-receptor population while the β_2-subtype accounts for the remaining 20% (Bristow et al., 1986; 1991). Generally, activation of myocardial β_1-adrenoceptors results in a positive inotropic and chronotropic response with little to no direct involvement from α-adrenoceptors.

Simultaneously, activation of centrally located α_{2A}-adrenoceptors decreases the sympathetic outflow and leads to a centrally mediated hypotensive effect (MacMillan et al., 1996; Altman et al., 1999), while inhibition of sympathetic transmission due to activation of presynaptic α_2-receptors in sympathetic ganglia may also contribute (McCallum et al., 1998). Consequently, it can be concluded that activation of α_2-adrenoceptors results in a biphasic blood pressure response: an initial hypertensive phase that is induced by α_{2B} receptors followed by a longer lasting decrease in blood pressure, predominantly mediated by both the baroreflex-mediated bradycardia and central sympatholysis.

2.2 Alpha$_2$-adrenoceptor agonists

Alpha$_2$-adrenoceptor agonists, such as xylazine, detomidine, medetomidine, dexmedetomidine, and romifidine, represent a very commonly used class of sedatives in veterinary clinical practice. They induce dose-dependent sedation, analgesia, and skeletal muscle relaxation that can be reversed via

administration of selective α_2-adrenoceptor antagonists. Except for xylazine, which is a thiazine derivative, all other α_2-adrenoceptor agonists and some antagonists have an imidazoline chemical structure. None of the clinically relevant α_2-adrenoceptor agonists is defined to be subtype specific. Alpha$_2$-adrenoceptor agonists are frequently combined with certain other sedatives, analgesics and/or anesthetics.

2.2.1 Xylazine

Xylazine (N-[2,6-dimethylphenyl]-5,6-dihydro-4H-1,3-thiazin-2-amine) was the first α_2-adrenoceptor agonist used in veterinary medicine. Xylazine has a 160 α_2/α_1-selectivity ratio for α-adrenoceptors (Virtanen et al., 1988), but it is 10–20 times more potent in ruminants than in other species (Kästner, 2006). The first published study in English on xylazine in animals was by Clarke & Hall (1969). Since then, numerous studies describing its pharmacodynamic and pharmacokinetic properties in various animal species have been published in the veterinary literature. In sheep, for instance, xylazine's sedative, analgesic, and pre-anesthetic effects have been extensively reported (Mitchell & Williams, 1976; Shokry et al., 1976; Hsu et al., 1987; 1989; Nolan et al., 1987; Kock, 1991; Papazoglou et al., 1994; Celly et al., 1997a; 1999a; Aminkov et al., 2002; Grant & Upton, 2004). Further, its combinations with agents such as ketamine and opioids have also been investigated in sheep (Nowrouzian et al., 1981; Green et al., 1981; Wright, 1982; Byagagaire & Mbiuki, 1984; Coulson et al., 1989; Lin et al., 1994; 1997; Hughan et al., 2001; Aminkov et al., 2002; Özkan et al., 2010; de Carvalho et al., 2016).

2.2.2 Detomidine

Detomidine (4-[2,3-dimethylbenzyl]-1H-imidazole) is an α_2-adrenoceptor agonist with an α_2/α_1 selectivity ratio of 260 (Virtanen et al., 1988), developed primarily to be used in horses and cattle. Therefore, there is a limited number of published reports regarding its clinical use in sheep. Nevertheless, detomidine's sedative effects have been reported in sheep after IV administration (Waterman et al., 1987), IM and IV (Singh et al., 1994) and constant rate infusion (de Moura et al., 2018). Additionally, its analgesic (Haerdi-Landerer et al., 2003) and cardiopulmonary (Celly et al., 1997a) effects were also investigated in sheep.

2.2.3 Romifidine

Romifidine (N-[2-bromo-6-fluorophenyl]-4,5-dihydro-1h-imidazol-2-amine), developed from clonidine (another α_2-adrenoceptor agonist seldomly reported in the veterinary literature), is a selective α_2-adrenoceptor agonist with an α_2/α_1 ratio of 340. Similarly to detomidine, romifidine is labeled mainly for sedation and premedication in horses, and it has all the usual properties of α_2-agonists. In one previous sheep study (Celly et al., 1997a), the degree of sedation and hypoxemia induced by IV romifidine (50 µg/kg) was comparable with that for xylazine (150 µg/kg IV), detomidine (30 µg/kg IV), or medetomidine (10 µg/kg IV).

2.2.4 Medetomidine and dexmedetomidine

Medetomidine (4-(1-(2,3-dimethylphenyl)ethyl)-1H-imidazole) is an equal racemic mixture of two optical enantiomers, dexmedetomidine and levomedetomidine. The effects of medetomidine are attributed to the dextroenantiomer, while levomedetomidine is considered to be pharmacologically inactive (MacDonald et al., 1991; Savola & Virtanen, 1991; Kuusela et al., 2000). Medetomidine and

dexmedetomidine are the most potent α_2-adrenoceptor agonists in veterinary practice, with an α_2/α_1 selectively ratio of 1620 reported for the racemic mixture (Virtanen et al., 1988).

2.2.5 ST-91

ST-91 (2-[(2,6-diethylphenylimino]-2-imidazolidine) is a hydrophilic derivative of clonidine, and it does not cross the blood-brain barrier (Kobinger & Pichler, 1975). Thus, it's mainly used as a model drug in experimental studies (Kästner, 2006). In sheep, ST-91 (3–30 µg/kg IV) did not produce any sedation, but it did induce a dose-related decrease in partial pressure of arterial oxygen (PaO_2) and heart rate (HR), also increasing mean arterial pressure (MAP) (Eisenach, 1988). Similarly, in halothane-anesthetized sheep receiving ST-91 (1.5–12 µg/kg IV), dose-dependent cardiopulmonary changes were observed (Celly et al., 1999b). Additionally, in conscious sheep, ST-91 (30 µg/kg IV) and clonidine (15 µg/kg IV) induced comparable cardiopulmonary responses (Celly et al., 1997b). Although the hypoxemic effects were less pronounced after ST-91 than after the centrally acting α_2-adrenoceptor agonists clonidine (Eisenach, 1988, Celly et al., 1997b) and medetomidine (Celly et al., 1999b), the induced pulmonary hypertension after ST-91 was longer-lasting than with medetomidine (Celly et al., 1999b). It is worth considering, however, that the doses used in these studies were not necessarily equipotent which makes direct comparisons between studies and the various agonists challenging.

2.2.6 Cardiopulmonary effects

Alpha$_2$-adrenoceptor agonists, regardless of their receptor affinity, induce various degrees of arterial hypoxemia in many ruminant species. The magnitude of the induced hypoxemia depends on factors such as the agonist dose and its route of administration, age, species, breed, and individual sensitivity, although sheep appear to be predisposed. Clinically, a wide range of respiratory symptoms have been reported, such as wheezy breathing with increased dyspnea associated with strong bubbling sounds on auscultation of the lungs (Uggla & Lindqvist, 1983), tachypnea (Hsu et al., 1987; 1989; Ko & McGrath, 1995; Celly et al., 1997a, b), decrease in respiratory frequency (Shokry et al., 1976; Mohammad et al., 1995; 1996), or no change (Borges et al., 2016).

In sheep, xylazine caused a severe reduction in PaO_2 after IV administration either to anesthetized (Aziz & Carlyle, 1978) or conscious animals (Celly et al., 1997a; Bacon et al., 1998). Likewise, IV medetomidine (5, 10, and 20 µg/kg) administered to conscious sheep led to a profound, dose-dependent decrease in PaO_2 (Bryant et al., 1996). In adult, conscious, spontaneously breathing sheep, xylazine (150 µg/kg), romifidine (50 µg/kg), detomidine (30 µg/kg), and medetomidine (10 µg/kg) induced a similar degree of hypoxemia after IV administration (Celly et al., 1997a). Similarly, IV xylazine (50 µg/kg) and detomidine (10 µg/kg) caused a pronounced hypoxemia in sheep, which was completely prevented by pre-treatment with idazoxan (100 µg/kg IV), an α_2-adrenoceptor antagonist (Waterman et al., 1987). Conversely, in one-month-old lambs sedated with medetomidine (30 µg/kg IV), neither significant changes in PaO_2 nor partial pressure of arterial carbon dioxide ($PaCO_2$) were evident; with the arterial hemoglobin oxygen saturation percentage (SaO_2) remaining above 90%. Neither IV atipamezole (30 or 60 µg/kg) nor yohimbine (1 mg/kg), both α2-adrenoceptor antagonists, altered the arterial oxygen tension (Ko & McGrath, 1995). Furthermore, for adult sheep sedated with medetomidine (30 µg/kg IM), PaO_2 remained within physiologically acceptable limits, albeit reduced from the baseline level (Kästner et al., 2003).

The hypoxemic effect of α_2-agonists in sheep is mainly mediated via the peripheral α_2-adrenoceptors (Celly et al., 1997b). However, the exact underlying mechanisms remain unresolved, although several theories have been proposed. Xylazine caused contraction of isolated sheep tracheal strips (Papazoglou et al., 1995) and increased airway pressure in halothane-anaesthetized sheep (Nolan et al., 1986a; Papazoglou et al., 1994). The authors speculated that the increased airway pressure was due to decreased dynamic compliance and/or increased airway resistance (Nolan et al., 1986a, Papazoglou et al., 1994). In addition, pulmonary edema formation as a result of platelet aggregation and pulmonary microembolism (Eisenach, 1988), pulmonary venoconstriction (Bacon et al., 1998), the release of inflammatory mediators due to the activation of intravascular pulmonary macrophages (Celly et al., 1999a) and increased hydrostatic pressure (Kästner et al., 2007) have been suggested to be the cause of hypoxemia. In sevoflurane-anesthetized sheep, dexmedetomidine markedly decreased dynamic compliance, and increased airway resistance, pulmonary shunt fraction and dead space ventilation (Kästner et al., 2005; 2007; Kutter et al., 2006). Furthermore, a severe bilateral edema was observed in computed tomography (CT) images of the ventral lung field, where the lung density was sharply increased 9–12 minutes after dexmedetomidine (2 µg/kg IV) administration in sevoflurane-anesthetized sheep (Kästner et al., 2007). Additionally, necropsy samples collected 10 minutes after dexmedetomidine administration revealed subpleural hemorrhage and the lungs appeared heavy with abundant amounts of blood drained from the excised tissue, while the trachea was filled with foamy fluid (Kästner et al., 2007). On histopathological examination, an eosinophilic alveolar edema associated with capillary congestion and extravasation of erythrocytes was detected (Kästner et al., 2007). Similar histopathological changes were also observed in sheep lungs 10 minutes after treatment with either xylazine (150 µg/kg IV) or ST-91 (30 µg/kg IV) (Celly et al., 1999a), and at 30 minutes after IV xylazine (200 µg/kg) (Bacon et al., 1998). An electron microscopic examination three minutes after xylazine was given revealed a mild pulmonary interstitial edema and after 10 minutes, extensive endothelial damage with interstitial and alveolar edema was evident (Celly et al., 1999a). Interestingly, with ST-91, alveolar edema and capillary endothelial damage were detected already after only three minutes (Celly et al., 1999a).

The cardiovascular effects of α_2-adrenoceptor agonists are very similar amongst various mammalian species (Greene & Thurmon, 1988; Murrell & Hellebrekers, 2005). Intravenous administration of α_2-adrenoceptor agonists typically lead to a biphasic blood pressure response, characterized by an initial hypertension followed by normo- or hypotension, accompanied by a profound bradycardia (Aziz & Carlyle, 1978; Savola, 1989; Vainio, 1989; Vainio & Palmu, 1989), reduced cardiac output (CO), increased central venous pressure (CVP) and SVR (Pypendop & Verstegen, 1998; Honkavaara et al., 2011; Rolfe et al., 2012). In sheep, IV administration of medetomidine (5, 10 and 20 µg/kg) resulted in decrease in HR and significant increase in MAP, the duration of which was dose-dependent. Cardiac output decreased after all three doses and remained reduced throughout a 60-minute observation period (Bryant et al., 1996). A target-controlled infusion of medetomidine (0.8, 1.6, 3.2, 6.4, and 12.8 ng/mL target concentrations) in awake sheep caused concentration-related reductions in HR and CO, and an increase in MAP (Talke et al., 2000). In sheep treated with ST-91, lacking central activity, the induced systemic and pulmonary hypertension were more pronounced and long lasting than after clonidine (Eisenach, 1988; Celly et al., 1997b) or medetomidine (Celly et al., 1999b) and occurred with a smaller dose than was required to produce pulmonary alterations (Celly et al., 1999b). In sheep, systemic hypotension (MAP < 60 mmHg) has not been reported after IV

administration of various α_2-adrenoceptor agonists. Although in one study (Bryant et al., 1996), some sheep may have had MAP values below 60 mmHg one hour after IV medetomidine (10 and 20 µg/kg), in most other investigations in sheep hypotension, expected to occur towards to end of the observational periods, was not observed (Celly et al., 1997a; de Carvalho et al., 2016; Borges et al., 2016).

The intensity of the cardiovascular response to α_2-adrenoceptor agonists probably depends on the route of administration. For instance, in humans, IV dexmedetomidine (2 µg/kg) caused a 10% decline in HR and 22% rise in MAP within five minutes of starting the infusion, but when the same dose was given IM, the changes in HR and MAP were less obvious (Dyck et al., 1993). Further, the dose also seems to account for the magnitude of induced cardiovascular effects in sheep. For example, only minor changes were observed in hemodynamics following IV administration of xylazine (150 µg/kg) (Doherty et al., 1986; Celly et al., 1997a), whereas marked cardiovascular changes were produced after a higher dose (500 µg/kg IV) in sheep (Aziz & Carlyle, 1978). However, the small number of animals used by Celly et al. (1997a) might also account for the lack of statistically significant differences, while in the other report by Doherty et al. (1986) the very low $PaCO_2$ could have attenuated the vasoconstrictive effects of xylazine, whereas the severe hypoxemia (PaO_2 < 40 mmHg) reported by Aziz & Carlyle (1978) might have had an opposite effect.

Reports on the effects of α_2-adrenoceptor agonists on the ovine pulmonary vasculature are somewhat inconsistent. In halothane-anesthetized, spontaneously breathing sheep, increasing the dose of medetomidine (0.5, 1, 2, and 4 µg/kg IV) were followed by statistically insignificant increases in mean pulmonary arterial pressure (MPAP), pulmonary vascular resistance (PVR), and pulmonary Arterial occlusion pressure (PAOP) when compared with saline-treated sheep (Celly et al., 1999b). Likewise, in conscious sheep receiving target-controlled infusions of medetomidine, no significant changes were detected in PAOP or MPAP, although PVR increased (Talke et al., 2000). Conversely, marked increases in MPAP and PAOP were observed following IV administration of dexmedetomidine (2 µg/kg) in mechanically ventilated, sevoflurane-anesthetized sheep (Kästner et al., 2007). Nevertheless, in a similar study setting, an equal dexmedetomidine dose did not cause significant changes in PVR or PAOP despite the significant increase in MPAP (Kutter et al., 2006). In general, the large inter-individual variation and small sample sizes might partially account for both the lack of statistically significant changes and some of the discrepancies in outcomes between apparently identically conducted investigations. For instance, in the aforementioned study, as only 4 sheep were studied, no significant differences were reached in PVR, although it increased from 85 ± 27 at baseline to 144 ± 82 dynes.sec/cm^5 (Kutter et al., 2006).

2.2.7 Central effects

The sedative effects of α_2-adrenoceptor agonists are mainly mediated via the α_{2A} receptors located in the *locus coeruleus* of the brainstem (Doze et al., 1989; Correa-Sales et al., 1992). The sedative properties of medetomidine were first demonstrated in mice and rats by Virtanen (1985) and in chicks by Savola et al. (1986). In domestic sheep, the use of medetomidine (25 µg/kg) was first reported as an IM combination with ketamine (1 mg/kg) by Laitinen (1990). The combination reportedly provided sufficient anesthesia for mandibular surgery, and with an induction time of 8.8 ± 3.6 minutes all the animals exhibited relaxation of limbs, jaws and neck muscles (Laitinen, 1990). Later, Mohammad et al. (1993) described the sedative and analgesic effects of medetomidine (40 µg/kg IM)

in Awassi sheep. Sedation was evident after nine minutes, recumbency occurred at 17 minutes and lasted for 58 minutes on average, respectively (Mohammad et al., 1993). In sheep receiving medetomidine (30 µg/kg IM) the sedative effects were apparent within 5 minutes and the maximum effect detected between 30-40 minutes after injection (Kästner et al., 2003). When the same dose was given IV to lambs, all animals became recumbent within 1–2 minutes and remained so for 73 ± 6.8 minutes (Ko & McGrath, 1995). In adult sheep, 40 µg/kg of IV medetomidine produced sternal recumbency 7 minutes after injection, although the authors suggested that this considerably long lag period was because some of the animals supported themselves against a pen wall for several minutes (Ranheim et al., 2000b). Elsewhere, premedication with IV dexmedetomidine (5 µg/kg) and medetomidine (10 µg/kg) had similar effects on both isoflurane-sparing and cardiopulmonary function in sheep undergoing orthopedic surgery (Kästner et al., 2001b). Similarly, no differences in isoflurane requirements after equipotent IM doses of dexmedetomidine (15 µg/kg) or medetomidine (30 µg/kg) were observed. However, HR was significantly higher with dexmedetomidine (Kästner et al., 2001a).

The antinociceptive effects of medetomidine and dexmedetomidine have been demonstrated in various animal species including rats (Vickery et al., 1988), dogs (Vainio et al., 1989; Tyner et al., 1997; Bennett et al., 2016), sheep (Muge et al., 1994), and cats (Ansah et al, 1998; 2000). When compared with buprenorphine, methadone, and flunixin meglumine, xylazine was the only agent that showed a significant analgesic effect in relieving acute pain induced by electric stimulation applied to lower hind limbs (Grant et al., 1996). Furthermore, xylazine (50 µg/kg IV) produced profound antinociception to both mechanical and thermal stimuli in healthy sheep (Nolan et al., 1986b; 1987), although its analgesic potency was significantly reduced in sheep suffering from chronic pain due to foot rot (Ley et al., 1991). Similarly, clonidine (6 µg/kg IV) produced antinociceptive activity to thermal and mechanical stimuli, which was more potent and longer lasting than that achieved with xylazine (50 µg/kg IV) in sheep (Nolan et al., 1987). Medetomidine, likewise, produced a dose-dependent antinociceptive effect to mechanical stimulation in sheep after IV administration, with all of the tested doses (1–7 µg/kg), except the lowest one, significantly raising the areas under the time-response curves when compared to the baseline or a saline control (Muge et al., 1994).

2.2.8 Other effects

Alpha$_2$-adrenoceptor agonists are known to induce hyperglycemia associated with hypoinsulinemia in several animal species (Metz et al., 1978; Eichner et al., 1979; Benson et al., 1984; Ambrisko & Hikasa, 2003; Restitutti et al., 2012; Pakkanen et al., 2018; Kallio-Kujala et al., 2018c). Stimulation of pancreatic α_{2A}-adrenoceptor inhibits insulin release and consequently increases plasma glucose (Fagerholm et al., 2004; 2008). In sheep, xylazine administration (160 µg/kg IV) led to a 4-fold increase in plasma glucose in the following 20 minutes (Muggaberg & Brockman, 1982), which was associated with hypoinsulinemia (Brockman, 1981; Muggaberg & Brockman, 1982). In some species, e.g. cattle and goats, glucose was also detected in the urine of xylazine or medetomidine-treated animals (Thurmon et al., 1978; Raekallio et al., 1994). For sheep, to the authors' knowledge, there is no available reports suggesting whether or not the α_2-adrenoceptor agonists-evoked hyperglycemia might be associated with glycosuria. Furthermore, medetomidine (40 µg/kg IV) induced two phases of hyperglycemia in sheep: the glucose concentration increased immediately after medetomidine, and then stabilized before a secondary surge, which reached its peak at 240 minutes. Reversal with

atipamezole (200 µg/kg IV), administered 60 minutes after medetomidine, prevented the later hyperglycemic phase (Ranheim et al., 2000a). In cattle, on the other hand, medetomidine (40 µg/kg IV) induced marked hyperglycemia, which peaked almost two hours after the treatment (Ranheim et al., 2000a).

Alpha$_2$-adrenoceptor agonists have been shown to increase urine production in several animal species, including cattle (Thurmon et al., 1978), horses (Thurmon et al., 1984), ponies (Trim & Hanson, 1986), goats (Raekallio et al., 1994), dogs (Burton et al., 1998, Saleh et al., 2005; Talukader & Hikasa, 2009), and cats (Murahata & Hikasa, 2012). In cattle, for instance, urine output was almost 10-fold higher during the first hour following IM xylazine 0.44 mg/kg and further increase to approximately18-fold in the second hour when compared to the control animals (Thurmon et al., 1978). Several factors have been suggested to be involved in this diuretic effect, including inhibition of antidiuretic hormone secretion from the pituitary gland (Roman et al., 1979; Cabral et al., 1998) and a consequent reduction in its plasma concentrations (Saleh et al., 2005; Talukader & Hikasa, 2009), inhibition of renin release in the kidneys (Smyth et al., 1987), inhibition of renal sympathetic activity (Menegaz et al., 2000), osmotic diuresis due to hyperglycemia (Thurmon et al., 1978; Burton et al., 1998), and inhibition of tubular sodium reabsorption (Gesek & Strandhoy, 1990). Administration of yohimbine and atipamezole antagonized medetomidine-induced diuresis in dogs (Talukder et al., 2009). On the other hand, in mice, vatinoxan failed to inhibit a dexmedetomidine-induced increase in voiding (Aro et al., 2015).

In sheep, xylazine dose-dependently decreased ruminal motility after both IV (Toutain et al., 1982) and IM (Mohammed et al., 1996) injections, although an early increase in food intake in sheep was observed (Mohammed et al., 1996). Furthermore, detomidine (10–40 µg/kg IV), xylazine (20–80 µg/kg IV), and clonidine (2.5–10 µg/kg IV) ceased both the primary and secondary contractions of the rumen, resulting in ruminal bloating which was effectively reversed by the α_2-adrenoceptor antagonists yohimbine (200 µg/kg IV) and tolazoline (400 µg/kg IV) in sheep (Ruckebush & Allal, 1987).

2.2.9 Pharmacokinetic properties

The studies describing the pharmacokinetics of α_2-adrenoceptor agonists in sheep are limited. Garcia-Villar et al. (1981) compared the pharmacokinetics of xylazine after IV and IM injections in four species: cattle, sheep, horses, and dogs. The data showed remarkably small interspecies differences, the elimination half-life ($T_{½}$) was about 23 minutes following both IV and IM administration of xylazine (1 mg/kg) in sheep (Garcia-Villar et al., 1981). After IM administration, the maximum plasma concentration (C_{max}) of 0.13 µg/mL was reached after 14.7 minutes (T_{max}), while the bioavailability was 40.8 ± 23.4% (Garcia-Villar et al., 1981). For medetomidine, the apparent volume of distribution (V_Z) was 2.7 L/kg following IV administration of 15 µg/kg and the $T_{½}$ was approximately 37 minutes, relatively short when compared to non-ruminant species and rationalized by a relatively high clearance (CL) of 57.5 mL/kg/min (Muge et al., 1996). Similar results were reported for sheep receiving a higher medetomidine dose (40 µg/kg IV) with a steady state volume of distribution (V_{dss}) of 1.8 L/kg, elimination half-life of approximately 35 minutes and clearance of 44.2 mL/kg/min (Ranheim et al., 2000b). Following IM administration of medetomidine (30 µg/kg) in sheep, the C_{max} was 4.9 ng/mL with T_{max} being 29.2 min, while the V_{dss}, $T_{½}$ and CL were 3.9 L/kg, 32.7 min, and 81 mL/kg/min, respectively (Kästner et al., 2003). The pharmacokinetics of dex- and

levomedetomidine have also been compared in sevoflurane-anesthetized sheep: the CL of the dextroisomer was significantly smaller (Kästner et al., 2006), possibly since levomedetomidine is less likely to affect its own disposition as it does not produce relevant cardiovascular effects (Dutta et al., 2000). Elsewhere, medetomidine was not detected in plasma following esophageal administration (15 µg/kg), suggesting a low bioavailability due to the high first pass metabolism in the liver after enteral administration (Hyndman et al., 2015).

2.3 Ketamine and its use with α_2-adrenoreceptor agonists in sheep

Ketamine (2-[2-chlorophenyl]-2-[methylamino]cyclohexan-1-one), a racemic mixture of S- and R-ketamine, "induces a dissociative or cataleptoid anesthesia in which the eyes remain open with a slow nystagmic gaze, varying degrees of hypertonus and purposeful or reflexive skeletal muscle movements often occur unrelated to surgical stimulation" (White et al., 1982). In sheep, this cataleptic state was first described by Thurmon et al. (1973), who used ketamine as a sole anesthetic agent. Irrespective of the dose (22–44 mg/kg) or route of administration (IV and IM), the eyes remained open with nystagmus and the movements of limbs were not associated with painful stimulations. The anesthetic and analgesic effects of IV ketamine in sheep seemed to be dose-dependent, as 2 mg/kg induced only ataxia and sternal recumbency while increasing the dose (5, 11.6 and 22 mg/kg IV) led to prolongation of anesthesia (Waterman & Livingston, 1978). In addition, salivation (Thurmon et al., 1973; Britton et al., 1974; Waterman & Livingston, 1978), mild regurgitation, and protrusion of tongue (Thurmon et al., 1973) were also observed. In contrast, in pregnant ewes receiving a smaller dose of ketamine (2 mg/kg IV) followed by continuous IV infusion, no salivation was noted (Taylor et al., 1972). Furthermore, in unpremedicated sheep and goats, ketamine (2 mg/kg IV) resulted in rapid recumbency associated with poor muscle relaxation, inconsistent analgesia, strongly maintained swallowing, pharyngeal and eructive reflexes, and poor conditions for orotracheal intubation (Green et al., 1981). Therefore, ketamine is typically not recommended to be used as a sole anesthetic in ruminants. The major metabolite of ketamine, norketamine, appears to retain about one-fifth to one-third of ketamine's activity in rodent models (Ebert et al., 1997; Holtman et al., 2008).

Co-administration of ketamine with agents that improve muscle relaxation, such as benzodiazepines and α_2-adrenoreceptor agonists, is recommended. For instance, premedication with xylazine (200 µg/kg IM) effectively reduced some of the undesirable effects of ketamine (22 mg/kg IV), such as muscle rigidity, ataxia, and insufficient suppression of reflexes in sheep (Nowrouzian et al., 1981). Likewise, satisfactory surgical conditions were achieved in sheep following pre-administration of xylazine (100 µg/kg IM) or diazepam (2 mg/kg IV) 10 and 15 minutes before ketamine (4 m/kg IV) respectively (Green et al., 1981). However, xylazine (100 µg/kg) in combination with ketamine (7.5 mg/kg) produced a longer lasting effective anesthesia than when the same dose of ketamine was combined with diazepam (0.375 mg/kg) IV (Coulson et al., 1989). Further, onset of anesthesia was significantly faster in sheep when ketamine (22 mg/kg) was combined IM with xylazine (200 µg/kg) rather than diazepam (0.4 mg/kg) (Özkan et al., 2010).

Consequently, ketamine is often combined with various α_2-adrenoreceptor agonists. In domestic sheep, medetomidine (125 µg/kg) and ketamine (2.5 mg/kg) resulted in rapid immobilization within three minutes after IM administration, characterized by lack of response to auditory or physical stimulation (Caulkett et al., 1994). Similarly, simultaneous IM administration of medetomidine (25

µg/kg) and ketamine (1 mg/kg) produced adequate anesthesia for mandible surgery as described earlier (Laitinen, 1990). After IV administration of medetomidine (20 µg/kg) and ketamine (2 mg/kg), sheep became sedated within 40 seconds, endotracheal intubation was easily performed after three minutes, and no limb rigidity or tremors were observed (Tulamo et al., 1995). Concomitant IM administration of ketamine (11 mg/kg) and xylazine (200 µg/kg) appeared to produce longer-lasting antinociception when compared with the IV route (Byagagaire & Mbiuki, 1984). Further, in non-domestic small ruminants, ketamine combined with various α_2-adrenoreceptor agonists is extensively used for reliable sedation and immobilization since the agonists alone appear insufficient (Jalanka & Roeken, 1990). Since the introduction of medetomidine into the veterinary market in the mid-1980s, its utility with ketamine for immobilization of non-domestic mammals has been extensively studied (Jalanka, 1988; 1989; 1990; Jalanka & Roeken, 1990; Tyler et al., 1990; Portas et al., 2003; Bush et al., 2004; Arnemo et al., 2005; 2011; 2013; Bouts et al., 2011; Evans et al., 2013).

The cardiopulmonary effects of ketamine are characterized by indirect cardiovascular stimulation as a result of its sympathomimetic effects mediated within the CNS (Ivankovich et al., 1974), inhibition of neuronal uptake of catecholamines by sympathetic nerve endings (Salt et al., 1979) and an inotropic effect on the myocardium (Tweed et al., 1972; Gelissen et al., 1996). Although direct vasodilation has also been reported (Altura et al., 1980), HR and arterial blood pressure tend to elevate due to an increase in the overall sympathetic outflow (Wong & Jenkins, 1974). Accordingly, ketamine has been reported to increase HR both after IV and IM injections (Thurmon et al., 1973) and blood pressure after IV administration in sheep (Waterman & Livingston, 1978). However, prior administration of sedative or anesthetic agents, which reduce sympathetic outflow from the CNS and/or decrease the amount of circulating catecholamines, such as α_2-adrenoreceptor agonists, could blunt the cardiovascular stimulatory effect of ketamine (Bidwai et al., 1975; Jackson et al., 1978; Bålfors et al., 1983; Reich & Silvay, 1989). In sheep, IV co-administration of xylazine (100 µg/kg) and ketamine (7.5 mg/kg) resulted in an immediate, transient reduction in SVR, and significant decreases in MAP, CO and Hb when compared with pre-treatment values (Coulson et al., 1989). However, after medetomidine (125 µg/kg) combined with ketamine (2.5 mg/kg) IM, HR, CI, and DO_2 were significantly decreased from the baseline while MAP (Caulkett et al., 1994; 1996), SVR, and PVR (Caulkett et al., 1996) increased, an outcome typical for α_2-adrenoreceptor agonists. Rapid administration of ketamine IV has also been shown to induce brief respiratory depression in sheep (Levinson et al., 1973; Waterman & Livingston, 1978) followed by a longer period of stimulation (Waterman & Livingston, 1978). Suggestive of marked hypoventilation in animals breathing room air, ketamine caused a dramatic decrease in PaO_2 and a significant increase in $PaCO_2$ immediately after IV bolus administration. However, PaO_2 returned to pre-treatment levels within 10 minutes and significantly exceeded it from 20 minutes onwards (Waterman & Livingston, 1978). While not observed in another study investigating IV ketamine in sheep (Thurmon et al., 1975), transient ventilatory suppression has been observed in other species as well, such as dogs (Gassner et al., 1974; Haskins et al., 1985), cats (Haskins et al., 1975), and humans (Waxman et al., 1980).

2.4 Alpha$_2$-adrenoceptor antagonists

The effects of α_2-adrenoreceptor agonists can be promptly reversed via administration of α_2-adrenoceptor antagonists, such as tolazoline, yohimbine, and atipamezole, many of which are licensed for veterinary use in several countries.

Yohimbine is an indole alkaloid that comes from a number of plant sources. It is a potent α_2-adrenoreceptor antagonist with a 40:1 selectivity ratio for α_2/α_1 adrenoceptors (Virtanen et al., 1989). Yohimbine has mainly been used to reverse xylazine-induced sedation in many species, including sheep (Hsu, 1981; 1983; Hsu & Shulaw, 1984; Hsu et al., 1987; 1989; Jensen, 1985; Jessup et al., 1985a, b; Kollias-Baker et al., 1993). However, it was less effective in reversing medetomidine-induced sedation than atipamezole in one-month-old lambs (Ko & McGrath, 1995) as discussed later. Moreover, IV yohimbine (125 µg/kg) 20 minutes after IV xylazine (150 µg/kg) and in laterally recumbent sheep, did not produce a clinically desirable reversal: it abolished the induced paradoxical respiratory patterns, but the improvement in PaO_2 took almost an hour (Doherty et al., 1986).

Tolazoline is a synthetic non-selective α-adrenoceptor antagonist (Casbeer & Knych, 2013) used to reverse sedation induced by α_2-adrenoreceptor agonists in ruminants (Takase et al., 1986; Hsu et al., 1987; 1989; Powell et al., 1998) and horses (Carroll et al., 1997; Hubbell & Muir, 2006). Tolazoline has higher affinity for sheep brainstem receptors than the other α_2-adrenoceptors, but compared with yohimbine and atipamezole, it has the lowest overall affinity for all α_2-adreneroceptor subtypes (Schwartz & Clark, 1998). When IV yohimbine (0.2 mg/kg) and tolazoline (2 mg/kg) were given five minutes after xylazine (0.3 mg/kg IV) to 6 ewes, tolazoline shortened the duration of sedation from 54 ± 5 minutes to 10 ± 2 minutes and reversed the decrease in PaO_2 within 10 minutes, whereas yohimbine did not significantly change the duration of either the induced recumbency (being 42 ± 8 minutes) or hypoxemia (Hsu et al., 1989). In another report by the same group (Hsu et al., 1987), using similar doses of tolazoline and yohimbine given IV 10 minutes after xylazine (0.4 mg/kg IV) to sheep, no significant differences were reported regarding sedation reversal, where both shortened the induced recumbency times from 41 minutes to 12 and 18 minutes on average, respectively (Hsu et al., 1987). The authors suggested that the difference in study protocols might account for this discrepancy, as the animals were confined to small pens in one study, but not in the other (Hsu et al., 1989).

2.4.1 Atipamezole

Atipamezole (5-[2-ethyl-1,3-dihydroinden-2-yl]-1H-imidazole) is the most specific α_2-adrenoreceptor antagonist, with a selectivity ratio of 8526 for α_2/α_1 (Virtanen, 1989; Virtanen et al., 1989). It reverses the centrally mediated sedative and analgesic effects induced by various α_2-agonists, for example in dogs (Clark & England, 1989; Vainio, 1990; Vainio & Vähä-Vahe, 1990; Granholm et al., 2007), horses (Raekallio et al., 1990; Luna et al., 1992; Yamashita et al., 1996; Di Concetto et al., 2007; Knych & Stanley, 2014), ruminants (Thompson et al., 1991; Arnemo & Søli, 1993a, b; 1995; Mohammad et al., 1995; Ko & McGrath, 1995; Ranheim et al., 1998; 1999; 2000b; Rioja et al., 2008), and non-domesticated species (Jalanka, 1988; 1989; 1990; Tsuruga et al., 1999; Bush et al., 2004; Arnemo et al., 2005; 2011). Atipamezole is widely used in veterinary clinical practice to antagonize medetomidine-induced sedation and to manage inadvertent over-dosages, but it is not currently licensed for food-producing animals. In sheep, less than two minutes following atipamezole administration (200 µg/kg IV) given 60 minutes after medetomidine (40 µg/kg IV), all the animals regained a standing posture (Ranheim et al., 2000b). Further, both the recovery time and time to walking were significantly shorter after reversal with atipamezole than with yohimbine in lambs sedated with medetomidine, as stated above (Ko & McGrath, 1995). More specifically, animals treated with yohimbine (1 mg/kg IV) 15 minutes after medetomidine (30 µg/kg IV) remained laterally

recumbent for about 12 minutes and did not walk unassisted until 16 minutes, on average, whereas with atipamezole (30 or 60 µg/kg IV) the animals walked unassisted within 3 minutes after reversal (Ko & McGrath, 1995). Atipamezole has a 100-fold higher affinity than yohimbine for the α_{2D} receptor subtype, the predominant one in the ovine brainstem, thus it seems more effective in antagonizing sedation in this species as speculated by Schwartz & Clark (1998). However, a relapse in sedation (resedation), drowsiness and somnolence were observed after reversal with atipamezole in dogs (Vainio & Vähä-Vahe, 1990; Vähä-Vahe, 1990) and cattle (Ranheim et al., 1998; 1999). Nevertheless, for up to seven hours after reversal with atipamezole, no resedation has been reported in sheep (Ranheim et al., 2000b).

In addition, atipamezole has been shown to be effective in reversing the peripheral effects of α_2-adrenoreceptor agonists as demonstrated in a number of species. Savola (1989) reported that atipamezole (10–300 µg/kg IV) completely restored HR and MAP when given during the most intense bradycardic and hypotensive phase induced by medetomidine in cats anesthetized with ether and chloralose (60 mg/kg IV). Later, Vähä-Vahe (1990) found that atipamezole reversed medetomidine-induced bradycardia in a dose-dependent manner in dogs. In sheep, atipamezole at a dose equal to five times that of the preceding medetomidine dose, reversed the reductions in HR, RR, and ruminal contractions (Mohammad et al., 1995). Moreover, in conscious, chronically instrumented sheep, when atipamezole was infused with a dose 2.5 times that of medetomidine, it significantly increased HR and reversed the increases in MAP, SVR, and $PaCO_2$. However, it failed to restore PaO_2 and PVR to pre-medetomidine levels (Talke et al., 2000). Additionally, pre-treatment with atipamezole prevented the xylazine-induced increase in airway pressure in halothane-anesthetized sheep (Papazoglou et al., 1994) and inhibited the contractile effect of xylazine on isolated sheep tracheal preparations (Papazoglou et al., 1995).

The pharmacokinetic properties of atipamezole (250 µg/kg IM) have been investigated both alone and 30 minutes after medetomidine (50 µg/kg IM) in dogs (Salonen et al., 1995). When used alone, the T_{max} and $T_{½}$ were 0.25 ± 0.21 and 0.95 ± 0.40 h respectively, C_{max} was 99.7 ± 35.1 ng/mL, Vz was 2.3 ± 1.12 L/kg, CL was 27.3 ± 4.9 mL/min/kg, and AUC was 156.7 ± 27.7 ng.h/mL (Salonen et al., 1995). Prior administration of medetomidine significantly altered the pharmacokinetics of atipamezole with T_{max} and T½ being 0.41 ± 0.34 and 1.2 ± 0.53 h respectively, C_{max} being 81.7 ± 16.7 ng/mL, CL was 23.5 ± 4.1 mL/min/kg, and AUC was 182.1 ± 35.1 ng.h/mL (Salonen et al., 1995). This was attributed to medetomidine-induced reduction in cardiac output and, consequently, hepatic blood flow as speculated by Salonen et al. (1995). On the other hand, atipamezole significantly altered the pharmacokinetics of medetomidine when used for reversal of sedation, particularly in ruminants. In sheep, for instance, atipamezole (200 µg/kg IV) given 60 minutes after medetomidine (40 µg/kg IV) significantly increased the plasma concentrations of medetomidine by 1.8–3.1 fold, which prolonged its detection time in plasma by 30 minutes when compared with sheep receiving saline (Ranheim et al., 2000b). Furthermore, atipamezole significantly decreased medetomidine's $T_{½}$ from 34.8 ± 7.3 to 21.8 ± 5.2 minutes, while no significant changes were detected in CL (44.2 ± 11.3 vs 38.5 ± 4.3 mL/ min/kg), V_Z (1.77 ± 0.26 vs 1.65 ± 0.19 L/kg), or AUC (953 ± 223 vs 1050 ± 115 ng.min/mL) (Ranheim et al., 2000b). The authors reported comparable results in reindeer (Ranheim et al., 1997), dairy calves (Ranheim et al., 1998) and cows (Ranheim et al., 1999), and they speculated that atipamezole may have displaced medetomidine from the highly perfused tissues such as CNS, kidneys, liver, and lungs, thus increasing its concentration in plasma.

2.4.2 Vatinoxan

Vatinoxan, previously known as L-659,066 and MK-467, (N-[2-[(2R, 12bS)-2'-oxospiro [1,3,4,6,7,12b-hexahydro-[1]benzofuro[2,3-a]quinolizine-2,5'-imidazolidine]-1'-yl] ethyl] methane-sulfonamide) with a selectivity ratio of 105:1 for α_2/α_1 adrenoceptors. It has a low lipophilicity (octanol:phosphate buffer partition coefficient of 1.3), which could explain why it penetrates the blood-brain-barrier (BBB) poorly, limiting its pharmacodynamic effects mainly to peripheral organ systems (Clineschmidt et al., 1988). However, other agents e.g. morphine and hydromorphone have a comparable octanol:phosphate buffer partition coefficient (Avdeef et al., 1996), but they do penetrate the BBB. Thus, the molecular weight, ionization degree, presence of active transport efflux, or protein binding characteristics might also contribute to vatinoxan's limited penetration through the BBB. For instance, the molecular weight for morphine is 285 g/moL while that of vatinoxan is 455 g/moL. In mice, P-glycoprotein (P-gp) is involved in regulating the extent of morphine transport across the BBB (Xie et al., 1999). The transcellular movement of vatinoxan was assessed in two cell line models transfected with human P-gp, and the results revealed that vatinoxan has no apparent permeability in the apical-basolateral direction suggesting that it is not a P-gp substrate (Bennett et al., 2017b). However, the occurrence of basolateral-apical transport indicates that vatinoxan may be a substrate for an unknown cellular efflux mechanism other than P-gp (Bennett et al., 2017b).

2.4.2.1 Cardiopulmonary effects

In a dose-dependent manner, IV vatinoxan slightly decreased MAP and increased HR, where the latter was strongly associated with increasing plasma noradrenaline levels in conscious rats (Szemeredi et al., 1989). The effects on HR and MAP were attributed to the inhibition of presynaptic α_2-adrenoreceptors and reflexive sympathetic activation due to blockade of α_2-receptors on arterial smooth muscle (i.e. increase in HR to compensate the effects of vasodilation on MAP) (Szemeredi et al., 1989). In humans, a transient, insignificant increase in blood pressure with no effect on HR was observed (Warren et al., 1991; Schafers et al., 1992). In sheep, pre-treatment with vatinoxan (264 µg/kg IV) failed to prevent the increase in MAP induced by the α_1-adrenoceptor antagonist methoxamine (75 µg/kg IV), while no effect on HR was reported (Bryant et al., 1998). In dogs, vatinoxan increases HR, CO, MPAP, DO_2 and oxygen consumption and decreases SVR after either a 10-minute IV infusion (Pagel et al., 1998) or when administered as a rapid IV bolus (Enouri et al., 2008). Similarly, Honkavaara et al. (2011) reported a transient increase in HR, CI, and DO_2 with a slight decrease in SVR, while MAP did not significantly differ from baseline values following IV administration of vatinoxan (250 µg/kg) in beagle dogs. Additionally, no significant changes were detected in PaO_2, $PaCO_2$, nor plasma lactate concentrations (Honkavaara et al., 2011). In cats, vatinoxan (600 µg/kg IV) increased HR and transiently increased MPAP while MAP, diastolic arterial pressure (DAP), and SVR decreased (Pypendop et al., 2017a). When the same dose was used IM in cats, HR transiently increased but no changes were recorded in MAP (Honkavaara et al., 2017b). Likewise, no changes were detected in MAP, PaO_2, or $PaCO_2$, while HR and RR increased significantly from the baseline for up to 30 minutes following IV vatinoxan (200 µg/kg) in horses (de Vries et al., 2016). Furthermore, in cats, 300 µg/kg IV did not induce any relevant changes in HR (Honkavaara et al., 2017a).

2.4.2.2 Sedative effects

In horses, vatinoxan (200 µg/kg IV) did not induce any significant changes in sedation scores (de Vries et al., 2016). Similarly in cats, vatinoxan (IV and IM) caused no significant change in sedation scores from baseline values (Honkavaara et al., 2017a, b). Recently, vatinoxan was found to increase the minimum alveolar concentration (MAC) of sevoflurane in dogs (Hector et al., 2017) and isoflurane in cats (Pypendop et al., 2019). The underlying mechanism of this effect remains unclear; however because the MAC-reducing effect of α_2-adrenoceptor agonists is exerted at the supraspinal level within the CNS (Kita et al. 2000), the authors speculated that vatinoxan may not be void of all central effects (Pypendop et al., 2019).

2.4.2.3 Other effects

A high dose of vatinoxan (30 mg/kg) induced hypoglycemia after intraperitoneal injection in mice (Durcan et al., 1991). In contrast, IV vatinoxan infusion up to 8 mg produced no consistent changes in blood glucose, insulin or plasma catecholamine concentrations in healthy human volunteers (Schafers et al., 1992). Similarly, there was no effect on fasting plasma glucose and insulin concentrations in human volunteers receiving two different doses of vatinoxan (15 and 30 mg) orally (Warren et al., 1991). In horses, vatinoxan (200 µg/kg IV) has no significant effect on plasma glucose concentrations (Pakkanen et al., 2018).

In horses, vatinoxan (200 µg/kg IV) did not cause detectable changes in intestinal motility, but three out of seven horses showed signs of restlessness and mild colic (kicking their pelvic limbs towards the abdomen). Two of these horses were reported to pass watery feces (de Vries et al., 2016). In dogs, one minute after IV vatinoxan (250 µg/kg), two out of eight dogs started to salivate, which lasted for a few minutes (Honkavaara et al., 2011).

2.4.2.4 Pharmacokinetic properties

There are no previous studies describing the pharmacokinetics of vatinoxan in sheep or other ruminant species. However, there exists a limited number of studies in other species. In dogs, the disposition data for IV vatinoxan (250 µg/kg) were: V_Z was 0.41 ± 0.13 L/kg, CL was 7.8 ± 3.4 mL/kg/min, and the $AUC_{0\text{-}60}$ was 26,600 ± 9100 ng/min/mL (Honkavaara et al., 2012). In cats, the following pharmacokinetic data after IV administration of vatinoxan (300 µg/kg) were reported: $T_{½}$, median (range), 122 (99–139) minutes, V_{ss} was 491 (379–604) mL/kg, CL was 3 (2–4.5) mL/min/kg, and the AUC was 100,665 (66,124–152,045) ng.min/mL (Pypendop et al., 2016). Similar results were obtained after a higher dose (600 µg/kg IV) with V_{dss} was 558 (501–605) mL/kg, $T_{½}$ was 97 (69–105) minutes, CL was 4.5 (3.8–6.3) mL/min/kg, and AUC was 133,466 (95,084–158,349) ng/min/mL (Pypendop et al., 2017b). It is worth mentioning that in the dog study (Honkavaara et al., 2012), the samples were collected only for 60 minutes after vatinoxan administration, which in part might explain the differences in pharmacokinetic variables from those reported for cats. On the other hand, following IM administration (600 µg/kg) of vatinoxan, the C_{max} was 913.9 (614–1436) ng/mL, T_{max} was 9.5 (1.3–19.4) minutes, the AUC was 102,450 (70,819–216,308) ng.min/mL, $T_{½}$ was 76.8 (64.2–98.8), and the bioavailability was 99.5% (52.6–148.7) in cats (Pypendop et al., 2017b). In horses treated with IV vatinoxan (200 µg/kg), the V_Z was 1189 ± 121 mL/kg, $T_{½}$ was 141 ± 28.6 minutes, CL was 6 ± 0.99 mL/min/kg, and the AUC_{inf} was 34,157 ± 5652 ng.min/mL (de Vires et al., 2016).

Overall, based on available studies, vatinoxan thus appears to have a lower volume of distribution and a slower clearance when compared to all clinically relevant α_2-adrenoreceptor agonists.

2.4.2.5 The use of vatinoxan with α_2-adrenoceptor agonists

In recent years, the effects of vatinoxan on the cardiopulmonary, sedative, and pharmacokinetic properties of various α_2-adrenoreceptor agonists have been described for several species (Table 1). However, in sheep only two previously published studies were located. Bryant et al. (1998) investigated the impact of vatinoxan (264 µg/kg IV) on medetomidine-induced cardiovascular changes in sheep and horses. Vatinoxan was proceeded and followed by methoxamine (75 µg/kg IV) and medetomidine (5 µg/kg IV) was given after cardiovascular variables (HR and MAP) returned to baseline levels or remained stable. The authors reported that vatinoxan attenuated the reduction in HR and the elevation in MAP caused by medetomidine (Bryant et al., 1998). Likewise, vatinoxan (250 µg/kg) alleviated all hemodynamic changes induced by dexmedetomidine (5 µg/kg) in sheep after IV co-administration (Raekallio et al., 2010). In addition, vatinoxan had no relevant effects on the degree of induced sedation in sheep (Raekallio et al., 2010). Similar findings were reported in other species with various agonists, such as in dogs (Honkavaara et al., 2008; Restitutti et al., 2011, Rolfe et al., 2012; Salla et al., 2014a), horses (de Vries et al., 2016; Tapio et al., 2018), cats (Pypendop et al., 2016; Honkavaara et al., 2017a), rats (Doze et al., 1989), and humans (Warren et al., 1991). Nevertheless, vatinoxan significantly reduced the total duration of sedation (Restitutti et al., 2011; Vainionpää et al., 2013b; Bennett et al., 2016). In standing horses sedated with medetomidine (7 µg/kg IV) with and without vatinoxan (140 µg/kg IV) and followed by 3.5 µg/kg/h medetomidine as a continuous rate infusion for 60 minutes, vatinoxan decreased the mean sedation scores during the first 20 minutes (Tapio et al., 2019). In contrast, vatinoxan significantly intensified the initial sedative effects of medetomidine, as reported in dogs (Restitutti et al., 2017; Kallio-Kujala et al., 2018a, b) and in cats with dexmedetomidine (Honkavaara et al., 2017b) following concurrent IM administration in the same syringe, which presumably was a consequence of the accelerated absorption as discussed earlier.

To the best of our knowledge, no previous studies have investigated IM co-administration of vatinoxan and medetomidine in sheep. Similarly, the effects of vatinoxan when combined with medetomidine and ketamine remain unexplored in this species. Furthermore, the interactions between vatinoxan and atipamezole have not been studied previously in sheep sedated with an α_2-adrenoreceptor agonists, with or without ketamine. Therefore, the following aims were defined for the present dissertation:

Table 1. Overview of the published studies on the interactions between vatinoxan and α_2-adrenoceptor agonists in certain animal species

Species	α_2-goinst/concurrent drugs (dose, route)	Vatinoxan (dose, route)/ technique	Primary outcomes	Reference
Dog	DEX (0.5 µg/kg/min, IV)	100 µg/kg, IV	VAT did not affect the arrhythmogenic effects of dexmedetomidine while the hypertensive bradycardic effects were significantly attenuated.	Hayashi et al., 1991
	DEX (5 µg/kg, IV)	100, 200, or 400 µg/kg, IV/30 min before DEX	DEX-induced increases in MAP, SVR, left ventricular pressure, coronary vascular resistance, and decreases in CO were abolished by VAT.	Pagel et al., 1998
	MED (10 µg/kg, IV)	200 µg/kg, IV/10 min before MED	VAT attenuated the induced cardiovascular changes; however, there was a decrease in HR, CI, DO_2, VO_2, and increase in SVR, CVP, PAOP, and ER from the baseline.	Enouri et al., 2008
	DEX (5 µg/kg, IV)	250 µg/kg, IV/in the same syringe	VAT did not significantly affect the quality of DEX-evoked sedation whilst significantly attenuated the induced bradycardia.	Honkavaara et al., 2008
	DEX (10 µg/kg, IV)	250, 500, or 750 µg/kg, IV /in the same syringe	Dose-dependently VAT alleviated the hemodynamic effects of DEX, the least alterations were seen with 50:1 dose ratio.	Honkavaara et al., 2011
	DEX (10 µg/kg, IV)	250, 500, or 750 µg/kg, IV /in the same syringe	No relevant differences in CSS from DEX alone after all VAT doses.	Restitutti et al., 2011
	DEX (10 µg/kg, IV)	500 µg/kg, IV /in the same syringe	DEX-elicited changes in plasma glucose, insulin, non-esterified free fatty acids, and lactate were prevented by VAT.	Restitutti et al., 2011
	MED (20 µg/kg, IM & 10 µg/kg, IV)	400 µg/kg, IM & 200 µg/kg IV/injected separately	After both IM and IV injections VAT significantly reduced the cardiovascular effects of MED without detectable impact on level of sedation.	Rolfe et al., 2012

Table 1. Continued

Species	α_2-goinst/concurrent drugs (dose, route)	Vatinoxan (dose, route)/ technique	Primary outcomes	Reference
Dog	DEX (10 µg/kg, IV)	500 µg/kg, IV/in the same syringe	VAT significantly reversed DEX-induced reduction in certain organs blood flow.	Restitutti et al., 2013
	MED (20 µg/kg, IV & IM)/BUT(0.1 mg/kg, IV & IM)	500 µg/kg, IV & IM/in the same syringe	In the presence of VAT the RT was lower while the superficial temperature measured by thermographic camera was higher with both routes.	Vainionpää et al., 2013a
	MED (10 µg/kg, IV)	250 µg/kg, IV/ in the same syringe	With VAT treatment HR, CI, DO_2 were significantly higher while MAP, SVR, CVP, and BIS were significantly lower than MED alone.	Salla et al., 2014a
	MED (20 µg/kg, IV & IM)/BUT(0.1 mg/kg, IV & IM)	500 µg/kg, IV & IM/in the same syringe	VAT addition significantly increased HR, CI, DO_2 and decreased aBP, SVR, CVP than MED/BUT alone after both routes.	Salla et al., 2014b
	MED (1.25 µg/kg, IV bolus) simultaneously CRI (8, 5.5, and 4 µg/kg/h)/0–20, 20–40, and 40–95 minutes, respectively.	CRI (100, 200, and 500 µg/kg/h)/5–35, 35–65, and 65–95 minutes after MED, respectively	At 1:50 infusion rate ration of MED:VAT, HR, MAP, CVP, and arteriovenous oxygen content differences were returned to their baseline values.	Kaartinen et al., 2014
	MED (20 µg/kg, IM)	400 µg/kg, IM/in the same syringe; 30 minutes later 100 µg/kg ATI, IM	VAT hastened the recovery after reversal with ATI. The hemodynamic function was better maintained in the presences of VAT and no resedation was observed.	Turunen et al., 2015
	MED (10 µg/kg, IV)	250 µg/kg, IV/ in the same syringe	VAT shortened the duration of MED sedation and significantly attenuated its analgesic effect. MED clearance was doubled in the presence of VAT, and $T_{½}$ and AUC were significantly deceased.	Bennett et al., 2016
	MED (20 µg/kg, IM)	200, 400, or 600 µg/kg, IM/mixed in the same syringe	MED Initial hemodynamic effects were not prevented by VAT; however, their duration was shortened. VAT enhanced MED absorption resulting in a faster sedation.	Restitutti et al., 2017

Table 1. Continued

Species	α_2-goinst/concurrent drugs (dose, route)	Vatinoxan (dose, route)/ technique	Primary outcomes	Reference
Dog	MED (loading, 4 µg/kg, IV; CRI, 4 µg/kg/h)/alfaxalone (loading, 2.4 mg/kg, IV; CRI, 3.6 mg/kg/h) 10 min after MED	Loading, 150 µg/kg, IV; CRI, 120 µg/kg/h/mixed in the same syringe with MED	VAT reversed MED-induced reduction in alfaxalone distribution and clearance. MED-induced decrease in HR, CI, and PaO_2; and increase in MAP, SVR, and $PaCO_2$ were ameliorated by VAT.	Bennett et al., 2017a
	MED (10 µg/kg, IV)/KET (0.5 mg/kg, IV) + MID (0.1 mg/kg, IV) 20 minutes after MED	50, 100, or 150 µg/kg, IV/in the same syringe with MED	A good cardiovascular stability was obtained when VAT 150 µg/kg was co-administered with MED.	Salla et al., 2017
	MED (20 µg/kg, IM)/BUT (100 µg/kg, IM)/MID (200 µg/kg, IM)	500 µg/kg, IM/all drugs mixed in the same syringe	VAT accelerated the absorption rate of BUT and MID and hastened the onset of peak sedation.	Kallio-Kujala et al., 2018a
	MED (0.5 mg/m2 IM)/BUT (10 mg/m2 IM)	10 mg/m2, IM/all drugs mixed in the same syringe	VAT alleviated medetomidine-induced bradycardia and increased the early plasma concentration of both MED and BUT which reflected by a deeper sedation for short period.	Kallio-Kujala et al., 2018b
	DEX (5 µg/kg, IV)/ glibenclamide (1 mg/kg, IV) or saline 10 min before DEX	250 µg/kg, IV/ mixed in the same syringe	VAT, however, augmented insulin release, it did not Potentiated the glibenclamide-evoked hypoglycemia.	Kallio-Kujala et al., 2018c
	MED (20 µg/kg, IM)/BUT (100 µg/kg, IM)/KET (4 mg/kg IV 20 min later)	500 µg/kg, IM/all drugs mixed in the same syringe	With VAT, CI was higher while MAP and SVRI was lower than treatment did not receive VAT.	Turunen et al., 2018
Cat	DEX (25 µg/kg, IV)	600 µg/kg, IV /in the same syringe	VAT moderately but significantly altered the pharmacokinetics of DEX through increasing its Cl and decreasing the AUC.	Pypendop et al., 2016
	DEX (25 µg/kg, IM)	600 µg/kg, IV/in the same syringe	All relevant DEX-induced cardiovascular changes were attenuated by VAT. A transient decrease in aBP was developed which did not lead to a hypotension.	Pypendop et al., 2017a

Table 1. Continued

Species	α_2-goinst/concurrent drugs (dose, route)	Vatinoxan (dose, route)/ technique	Primary outcomes	Reference
Cat	DEX (25 µg/kg, IM)	600 µg/kg, IM/in the same syringe	DEX T_{max} was significantly by addition of VAT, while the C_{max} and Cl were significantly increased.	Pypendop et al., 2017b
	DEX (25 µg/kg, IV)	75, 150, 300, or 600 µg/kg, IV/in the same syringe	Except with the higher VAT dose (600 µg/kg), HR decreased significantly below the baselines after all dose combinations. The quality of sedation did not impacted by VAT; however, the highest dose significantly reduced the sedation duration.	Honkavaara et al., 2017a
	DEX (25 µg/kg, IM)	300, 600, or 1200 µg/kg, IM/in the same syringe	With VAT 600 and 1200 µg/kg, HR only decreased below the baselines during the first 3 minutes post-injection. While VAT prevented the early changes vasopressor of DEX, MAP significantly decreased with all doses it decreased intensified DEX early sedative effects, but shortened the duration of its central action.	Honkavaara et al., 2017b
	DEX (25 µg/kg, IM)	600 µg/kg, IM/in the same syringe	Pulse rates remained significantly below the baseline throughout the 360 minutes observation period regardless to the addition of VAT. CVP, aBP, MPAP, and PAOP initial increased, but with VAT returned rapidly to baselines.	Siao et al., 2017
	DEX (10 ng/mL, TCI)	3 serially increasing doses (15, 30, and 60 µg/kg, IV)	Each of VAT doses significantly decreased aBP, and increased HR. Hypotension (MAP < 60 mm Hg) was developed after VAT 30 and 60 µg/kg albeit CI and SVR were returned to baseline values.	Martin-Flores et al., 2017
	DEX (0–40 ng/mL, TCI) /isoflurane- anesthetized	Target infusion (4 ng/mL)	In a plasma concentration-dependent manner, VAT significantly decreased MAC sparing effect of DEX and increased the baseline MAC.	Turunen et al., 2015
Horse	MED (5 µg/kg, IV)	264 µg/kg, IV preceded and followed by methoxamine (75 µg/kg, IV) then MED given after CVS returned to BL values	Pretreatment with VAT significantly reduced MED-induced hypertension and attenuated the induced decrease in HR.	Bryant et al., 1998

Table 1. Continued

Species	α_2-goinst/concurrent drugs (dose, route)	Vatinoxan (dose, route)/ technique	Primary outcomes	Reference
Horse	DET (10 µg/kg, IV)	250 µg/kg, IV/in the same syringe	VAT attenuated DET effects on HR and CVP. The maximal sedation scores did not impacted by the presence of VAT. DET-induced intestinal hypomotility was prevented by VAT. DET CL and V_Z were increased by VAT while the AUC was decreased. $PaCO_2$ were ameliorated by VAT.	Vainionpää et al., 2013b
	DET (20 µg/kg, IV)/BUT (20 µg/kg, IV)/isoflurane-anesthetized	200 µg/kg, IV/in the same syringe	Addition of VAT significantly reduced the induction time, and increased HR, CI, and deceased MAP and SVR. DET plasma concentrations and AUC were significantly reduced in the presence of VAT.	Pakkanen et al., 2015
	ROM (80 µg/kg, IV)	200 µg/kg, IV/in the same syringe	VAT attenuated ROM-induced intestinal hypomotility without affecting the quality of induced sedation. Romifidine $T_{½}$, V_Z, and CL were significantly increased by VAT while AUC decreased.	de Vries et al., 2016
	DEX (premedication, 3.5 µg/kg, IV; CRI, 7 µg/kg/h), or XLY (premedication, 0.5 mg/kg, IV; CRI, 1 mg/kg/h)/isoflurane-anesthetized	250 µg/kg, IV/110 minutes later	Following VAT injection colon, jejunum, and stomach blood flow were reduced significantly without relevant changes in tissue oxygen saturation. MAP, SVR were significantly decreased, while CI was significantly increased. No significant changes detected in CVP, PAP, and alveolar dead space.	Wittenberg-Voges et al., 2018
	DET (20 µg/kg, IV)	150 µg/kg, IV/10 minutes after DET n the same syringe	Less than 5 minutes post VAT injection, DET-induced bradycardia was reversed.	Tapio et al., 2018
	ROM (80 µg/kg, IV)	200 µg/kg, IV/in the same syringe syringe	VAT significantly reduced ROM-induced hyperglycemia, however, plasma glucose remained higher than the BL. No differences were detected in insulin, cortisol, and adrenocorticotropic hormone concentrations.	Pakkanen et al., 2018
	DEX (premedication, 3.5 µg/kg, IV; CRI, 7 µg/kg/h)	130 µg/kg IV; CRI, 40 µg/kg/h	VAT ameliorated intestinal tissue damage after ischemia and reperfusion.	Kopp et al., 2018

Table 1. Continued

Species	α_2-goinst/concurrent drugs (dose, route)	Vatinoxan (dose, route)/ technique	Primary outcomes	Reference
Horse	DEX (premedication, 3.5 µg/kg, IV; CRI, 7 µg/kg/h)	130 µg/kg IV; CRI, 40 µg/kg/h	DEX plasma concentrations did not affected by VAT. DO_2, SaO_2, and CO were significantly higher with VAT, while ER, venous admixture, alveolar dead space and alveolar-arterialoxygen were lower. VAT improved the tissue perfusion.	Neudeck et al., 2018
Rat	Clondine		VAT (from 0.3 to 30 mg/kg IV) did not significantly altered clonidine-elicited mydriasis, while pretreatment with VAT caused a dose-dependent antagonism.	clineschmidt et al., 1988
	DEX (20 µg/kg, IP) or (180 µg/kg, IP)	1 mg/kg, IP; or 9 mg/kg, IP/15 minutes before DEX	VAT only blocked DEX-evoked analgesic effect in neuropathic rats without affecting neither induced sedation nor analgesia in normal ones.	Poree et al., 1998
	DEX (10 µg/kg, IV)	1 mg/kg, IV/10 minutes before DEX	Pretreatment with VAT did not affect visceral nor somatic antinociceptive effects of DEX.	Ulger et al., 2009
Mice	MED (30 µg/kg, IV)	3 mg/kg, IV/10 minutes before MED	VAT significantly attenuated MED-evoked bradycardia; however, it has a no effect on the induced hypotension. Also, it potentiated glibenclamide-induced hypoglycemia.	Ruohonen et al., 2015
Bharals	MED (80 µg/kg, IM)/KET (1.5 mg/kg, IM)	20 times of MED dose mixed in the same dart and given IM	VAT alleviated the bradycardia and hypertension induced by medetomidine. When the measurements done (25–30 minutes post-dart), HR was 50 vs 80 bpm, and MAP was 130 vs 117 mmHg, without and with VAT; respectively.	Sainmaa et al., 2018
Markhors	MED (80 µg/kg, IM)/KET (1.5 mg/kg, IM)	117–297 µg/kg, IV/ almost 20 minutes after dart; ATI (5 times MED dose) given IM 33–75 minutes after dart	Post-treatment with VAT significantly increased the HR (3/10 animals after VAT vs 6/10 before VAT), and alleviated hypertension (5/10 after vs 10/10 before) without inducing hypotension. No changes were detected in RR, RT, blood biochemical indices, or recovery time.	Sainmaa et al., 2019

aBP, arterial blood pressure; ATI, atipamezole; BIS, bispectral index; BL, baseline; BUT, butorphanol; CRI, continous rate infusion; CSS, composite sedation score; CVS, cardiovascular system; DET, detomidine; DEX, dexmedetomidine; ER, oxygen extraction ratio; IP, intrapretoneal; KET, ketamine; MAC, minimal alveolar concentration; MED, medetomidine; MID, midazolam; PAOP, pulmonary artery occlusion pressure; ROM, romifidine; RT, rectal temperature; TCI, target-controlled infusion; VAT, vatinoxan; VO_2, oxygen consumption; XYL, xylazine.

3 AIMS OF THE STUDY

1. To investigate the cardiopulmonary effects of vatinoxan in conscious sheep treated with medetomidine or medetomidine-ketamine, with special reference to concomitant IM administration and interactions with atipamezole (studies I and II).

2. To determine the effect of vatinoxan on the exposure to medetomidine following IM co-administration (studies I–III).

3. To explore the impact of vatinoxan on plasma concentrations of atipamezole (studies I–III), ketamine and norketamine (study I), and dexmedetomidine (study IV).

4. To evaluate the impact of vatinoxan on medetomidine-ketamine and medetomidine-induced sedation and reversal with atipamezole (studies I–III).

5. To characterize the influence of vatinoxan on dexmedetomidine-induced pulmonary and cardiovascular changes in sevoflurane-anesthetized sheep (study IV).

4 MATERIALS AND METHODS

4.1 Animals

Nine adult female sheep (4 Texel and 5 Crossbred [Texel, Dorset, and Gotland] sheep), aged between 1 and 5 years during the experiments, were used in these studies. For study I all nine sheep were included, whereas for studies II and III eight of them, and for study IV seven animals were used. Approximately one month before the beginning of study I, the right carotid arteries were surgically exteriorized into a subcutaneous position under general anesthesia using the surgical method described previously for standing horses (Tapio et al., 2017). The animals were premedicated with IV detomidine (50 µg/kg), butorphanol (0.5 mg/kg), and ketamine (0.5–1 mg/kg), anesthesia was then induced with IV propofol until effect and maintained with isoflurane. All studies were approved by the National Experiment Board of Finland (License number: ESAVI/9394/04.10.07/2015). The sheep were housed as a single group with free access to hay and water. Only before general anesthesia (study IV) was hay, but not water, withdrawn for 24 hours. The animals were considered healthy based on clinical examination, complete blood counts and serum chemistry analyses.

4.2 Instrumentation

In studies I and II the animals were maintained upright in a padded sling. A single-lumen polyethane central venous catheter (Cavafix Certo, B. Braun, Melsungen, Germany, 18 G; study I and 14 G; study II) was inserted via the left jugular vein into the thoracic vena cava for measuring the central venous pressure (CVP) and collecting blood samples (study II). An 18-gauge venous cannula (Terumo Europe, Leuven, Belgium) was then placed in the right jugular vein for lithium injection and a 20-gauge arterial catheter (Becton Dickinson, UT, USA) was inserted into the elevated carotid artery. The catheters were later connected to pressure transducers (Gabarith PMSET, Becton Dickinson, UT, USA). Local anesthesia (0.5 mL of Lidocaine 20 mg/mL; Orion, Turku, Finland) was subcutaneously injected at each site prior to insertion of the catheters.

For study IV, the cephalic vein was cannulated (18 G) and anesthesia was induced with IV administration of propofol to effect. Following orotracheal intubation, anesthesia was maintained with sevoflurane (SevoFlo, Abbott Laboratories, IL, USA) in 50% inspired oxygen (Perseus A 500, Dräger, Germany). A triple-lumen Swan-Ganz catheter (7 Fr, 110 cm, Edwards Lifesciences, CA, USA) was introduced via a 10 G 3 Teflon introducer (Becton Dickinson, UT, USA) via the left jugular vein and the tip was advanced under pressure guidance into the pulmonary artery. The carotid artery was catheterized similar to studies I and II and a 16 G cannula was placed in the right jugular vein for drug administration. All catheter placements were performed aseptically. Study III was conducted without any instrumentation.

4.3 Study design

All studies were crossover designs with randomly allocated treatments using balanced Latin square design. The washout period between treatments was at least 14-days (studies I–III), and 20-days for study IV. A summary of studies, treatments, and doses is presented in Table 2. The investigators assessing sedation (studies I–III), monitoring cardiovascular data (studies I–IV), and evaluating the CT-scans (study IV) were masked to the treatments.

4.4 Drugs and doses

Medetomidine (Dorbene 1 mg/mL, Syva Laboratories S.A., Spain), dexmedetomidine (Dexdomitor 0.1 mg/mL, Orion Pharma, Turku, Finland), vatinoxan (Vetcare Ltd., Mäntsälä, Finland), ketamine hydrochloride (Ketador vet. 100 mg/mL, Richter Pharma AG, Wels, Austria), and atipamezole (Alzane 5 mg/mL, Syva Laboratories) were used. Vatinoxan was provided in powder form. Prior to each treatment, it was dissolved in medetomidine (studies II and III), medetomidine and ketamine, and isotonic saline (study I), or isotonic saline only (study IV). Medetomidine and/or medetomidine-ketamine, with or without vatinoxan and/or isotonic saline, were injected IM in the same syringe (studies I–III), atipamezole (ATI) was administered IM in the contralateral muscle 60 (study I) or 30 (studies II and III) minutes after the initial treatment. In study IV, vatinoxan was given as an IV bolus ten minutes (T-10) before dexmedetomidine, which was diluted in isotonic saline to a final volume of 20 mL and administered IV over 30 seconds via the jugular vein (Table 2).

Table 2. Summary of studies.

Study	Animals used	Treatments	Outcomes
I	9	a. MED 30 µg/kg + KET 1 mg/kg IM (Med-Ket) b. Med-Ket + 150 µg/kg VAT IM (Med-Ket-Vat150) c. Med-Ket + 300 µg/kg VAT IM (Med-Ket-Vat300) d. Med-Ket + 600 µg/kg VAT IM (Med-Ket-Vat600) ATI (150 µg/kg, IM) 60 minutes later to all treatments	Cardiopulmonary Plasma concentrations Clinical sedation Atipamezole reversal
II	8	a. Medetomidine 30 µg/kg IM (MED) b. MED + 300 µg/kg VAT IM (Med-Vat) c. MED followed by ATI at T30 (150 µg/kg, IM) d. Med-Vat followed by ATI at T30 (150 µg/kg, IM)	
III		a. Medetomidine 30 µg/kg IM (MED) b. MED + 300 µg/kg VAT IM (MED+VAT) ATI (150 µg/kg, IM) 30 minutes later to all treatments	Clinical sedation Atipamezole reversal
IV	7	a. Dexmedetomidine 3 µg/kg IV (DEX) b. DEX preceded by VAT 150 µg/kg IV (VAT+DEX)	Cardiopulmonary CT imaging, BAL Ventilatory mechanics

4.5 Cardiopulmonary monitoring

A continuous lead II electrocardiogram, invasive arterial and central venous pressures (studies I, II, and IV) and pulmonary arterial pressures (study IV) were recorded (S/5 Compact Critical Care Monitor, Datex-Ohmeda, Helsinki, Finland). The invasive pressure transducers were tested against a mercury manometer and zeroed to atmospheric pressure prior to baseline measurements. The height at the shoulder joint was used as the zero reference point for standing blood pressure measurements (studies I and II), and the level of the CT-table for sternal recumbency (study IV). Samples for arterial blood gas analysis were taken via the carotid arterial catheter into pre-heparinized syringes, stored in iced water and analyzed immediately (studies I, II, and IV). In study II, central venous blood samples

were similarly collected from the CVP catheter for blood gas analysis, whereas in study IV mixed venous blood samples were collected from the distal port of the pulmonary arterial catheter. The arterial hemoglobin content, pH, PaO_2, $PaCO_2$, glucose, electrolyte and lactate concentrations were measured and bicarbonate (HCO_3^-) calculated using the following blood gas analyzers: Epoc (Epocal Inc, Ottawa, ON, Canada; study I), GEM 4000 (GEM Premier 4000, Bedford, MA, USA; study II), and ABL 855 (Radiometer, Copenhagen, Denmark; study IV). In study I, Hb contents were analyzed using a hematology system (ADVIA 2120i, Siemens Healthcare GmBH Germany). Respiratory rates (RR) were calculated by observing chest wall movements (studies I and II). In study III, pulse rates were counted by palpating the carotid pulse wave, whereas in study IV, tidal volume (V_T), end-tidal carbon dioxide ($ETCO_2$), respiratory rate (f_R), fraction of inspired oxygen (FiO_2), end-tidal sevoflurane, total dynamic compliance (C_{dyn}), and peak inspiratory pressure (PIP) were obtained from the anesthesia monitor (Perseus A 500, Dräger, Germany).

Cardiac output was measured using the lithium dilution method (LidCO Plus Hemodynamic Monitor, LidCO Ltd., Cambridge, UK), as previously described in dogs (Mason et al., 2001) and sheep (Raekallio et al., 2010). Briefly, the lithium sensor was prepared as described in the operation manual and attached to the side port of a 3-way stopcock connected to the arterial catheter. To begin the cardiac output measurements, the flow regulator was activated at a rate of 4 mL/min and a standard dose of 0.075 mmoL lithium chloride (previously placed into an extension set) was flushed with 20 mL of saline via the jugular cannula. The LidCO computer requires blood hemoglobin and sodium concentrations to produce accurate CO values, and standard concentrations of 10 g/dL and 140 mmol/L were initially used for hemoglobin and sodium, respectively. Later, they were corrected with measured values obtained from simultaneously taken arterial blood samples using a formula provided by LidCO Ltd.

Standard formulae were used for calculation of cardiopulmonary indices (Boyd et al., 1991, Haskins et al., 2005). A formula specific to sheep (Maginniss et al., 1986) was used for calculating arterial (SaO_2; studies I and II) and venous oxygen saturation (SvO_2; study II). In study IV, SpO_2 was obtained directly from the pulse oximeter (Nonin PalmSAT 2500 series, MN, USA).

4.6 Assessment of sedation

The sedation was subjectively assessed using a visual analogue sedation score (VAS) with 0 indicating no sedation and 10 deep sedation (studies I–III). Additionally, in study III a descriptive sedation scale (Kästner et al., 2003) was used, with 0 indicating standing, alert and normal behavior while 10 indicated lateral recumbency with no movement. The sedation was evaluated before giving the initial medetomidine-containing treatments and at intervals thereafter for two hours (studies I and II) and up to 5 hours (study III). Moreover, in study III, the time (minutes) elapsed from drug injection until first signs of sedation, the time to deep sedation (recumbent and unable to support the head), and the time from atipamezole administration until recovery (standing-up) were recorded. The area under the time-sedation curves (AUC) were calculated using the trapezoidal method from baseline to 70 minutes (study I) and from baseline to 30 minutes (study III) for each sheep. Resedation was assessed subjectively by observing signs of reduced alertness and activity after the initial recovery.

4.7 Drug concentrations in plasma

Blood samples were collected into EDTA tubes from the carotid artery catheter in studies I, II and IV, whereas in study III they were obtained from the jugular veins by direct venipuncture. Samples were placed in iced water, centrifuged at 3000 x g for 15 minutes and the plasma was stored at -20 °C until analyzed. A summary of the drugs analyzed in each study is presented in Table 3. All analyses were performed with liquid chromatography mass spectrometry (LC-MS/MS).

Table 3. Summary of individual drug analyses.

Study	Analyzed drugs
I	Dexmedetomidine, levomedetomidine, ketamine, norketamine, vatinoxan & atipamezole
II	Dexmedetomidine, levomedetomidine, vatinoxan & atipamezole
III	
IV	Dexmedetomidine & vatinoxan

The concentrations of dex- and levomedetomidine (reference standard: racemic medetomidine, TRC, Toronto, Ontario, Canada) in EDTA plasma were calculated after solid-phase extraction (Sep-Pak®tC18 96 well extraction plates, Waters Corporation, Milford, MA, USA) with racemic d3-medetomidine as the internal standard. After chiral separation (Chiralpak® AGP column, 4 x 150 mm, 5 µm, Chiral Technologies Europe, Illkirch, France) with 10 mM ammonium acetate (pH 4.5) and acetonitrile containing 0.1 % formic acid as solvents, quantitative detection was performed in multi-reaction monitoring mode (MRM) with a triple quadrupole mass spectrometer (triple quadrupole mass spectrometer, 4000QTrap, MDS Sciex, Concord, Ontario, Canada). The chromatograms were processed with industry-standard software (Applied Biosystems/MDS Sciex software, Analyst version 1.6.1). The linear concentration range was from 0.10 to 10.0 ng/mL. The inter-assay accuracy of the quality control samples (at 0.225, 1.0, 8.0 and 50 ng/mL) ranged from 94.4 to 99.8 % (dexmedetomidine) and 92.5 to 99.2 % (levomedetomidine).

For ketamine, norketamine, vatinoxan and atipamezole, 50 µL plasma aliquots were treated with in-well protein precipitation on a Sirocco plate (Sirocco plate, Waters Co., Milford, MA, USA) with 250 µL of internal standard solution in acetonitrile (containing 100 ng/mL propranolol and 20 ng/mL chlorpromazine). After vigorous mixing for 5 min, the samples were centrifuged for 20 min at 4000 RPM. 50 µL aliquots of the sample supernatants were transferred on to UPLC 96-well plates, diluted with 450 µL of 20 % acetonitrile in water and analyzed. Reference samples were prepared in drug-free sheep plasma at analyte concentrations of 0.02 to 20 000 ng/mL. Quality control samples were analyzed at concentrations of 0.2, 2, 20, 200 and 2000 ng/mL. The detection limit was 1 ng/mL for vatinoxan, ketamine, and atipamezole and 0.5 ng/mL for norketamine. The quantitation range for vatinoxan was 1–10,000 ng/mL (studies I–III) and 2–2000 ng/mL (study IV), while for ketamine and norketamine they were 1–10,000 and 0.5–2000 ng/mL, respectively, and 1–10,000 ng/mL for atipamezole. The accuracy (%) for vatinoxan, ketamine, norketamine, and atipamezole were 96–105, 92–106, 93–103, and 91–108, and the precision (%) were 6.8–13, 1.3–19.5, 0.5–19.9, and 0.4–11.3, respectively.

The pharmacokinetic variables in study I for $AUC_{0–50}$ were calculated using the trapezoidal method, while C_{max} and T_{max} values were derived from the plasma concentration data.

4.8 Computed tomography (CT)

In study IV, CT was performed (GE Lightspeed VCT 64, GE Healthcare, Waukesha, WI, USA). The lung parenchyma caudal to the level of heart was scanned at 140 kV, at an mA noise index of 18.0 (min 120 mA/max 710 mA), and slice thickness of 0.625 mm, with a rotation time of 0.4 sec and detector pitch at 0.984. The CT scanner was calibrated daily according to the hospital's protocol. The cross-sectional areas (cm^2) of the caudal pulmonary artery and vein were measured in the right lung field. Two regions of interest (ROI) of 0.5 cm^2 were drawn on this same image as described previously (Kästner et al., 2007): one positioned dorsally close to the periphery of the lung parenchyma, and the other ventrolaterally to the main bronchus (Figure 4A), and the CT values of each ROI were calculated as Hounsfield units (HU). The measurements for each ROI were conducted in triplicate by an investigator masked to the treatments, for a total of three times with at least a one week interval.

4.9 Bronchoscopy and bronchoalveolar lavage (BAL)

In study IV, bronchoscopy and bronchoscopy-guided BAL were performed via the endotracheal tube approximately five minutes after the last CT scan. Fifty milliliters of saline was infused through the work channel of the bronchoscope (Olympus GIF-N180, Olympus Europa GmbH, Japan) into the right dorsal and right accessory lung lobes, and the fluid was immediately withdrawn and placed on ice. The BAL fluid was then filtered through a sterile gauze, after which the quantity of white blood cells was calculated and differential counts were performed from cytocentrifuge smears stained with May-Grünwald Giemsa.

4.10 Statistical analyses

The cardiopulmonary variables were compared within and between treatments with a mixed model repeated measures ANCOVA, followed by post-hoc tests where appropriate. Treatment comparisons for changes from baseline by time and changes within treatment were computed from the same model with contrast (study I) and Holm–Bonferroni post-hoc corrections were used where applicable (studies II–IV). Pharmacokinetic variables and plasma drug concentrations between treatments and/or studies at certain time-points were analyzed with one-way ANOVA followed by the Tukey post hoc test and Student's *t*-test as appropriate (studies I and II). In study I, the trapezoidal method was used to calculate the areas under the time-sedation score curves from baseline to 70 minutes for each sheep and the values were analyzed with a repeated-measures ANOVA. Equivalence among treatments was to be accepted when the 95% confidence interval of the geometric mean ratio was within the reference limits (0.8 and 1.25). In studies II and III, the sedation scores were analyzed with Friedman's test for the time effect within each treatment while comparisons between treatments were analyzed with Kruskal–Wallis and Mann–Whitney U tests where applicable. The CT measurements and BAL fluid differential white blood cell counts were analyzed with the Wilcoxon Signed-Rank test. For all studies, $P < 0.05$ was considered statistically significant. The analyses were computed using IBM SPSS Statistics version 24.0 for Windows (IBM Corp., Armonk, NY, USA) and SAS for Windows, version 9.3, (SAS Institute Inc., Cary, NC, USA).

5 RESULTS

5.1 Cardiopulmonary effects

The data for cardiopulmonary variables from all studies are summarized in Tables 4–6 and Figures 1–3. In study I, addition of vatinoxan prevented the initial medetomidine-induced increase in SAP, DAP, MAP, and CVP, and subsequent blood pressures were also significantly below baseline values after all doses of vatinoxan (Table 4). Without vatinoxan, HR remained significantly below baseline until reversal with atipamezole (Figure 1). Similarly, in study II, HR decreased after MED and, even after atipamezole, remained below the baseline values (Figure 2). Cardiac output decreased after MED alone and remained below baseline throughout the observational period. Atipamezole reversed this medetomidine-induced decrease (Table 4 and 5). Systemic vascular resistance increased after MED alone and remained significantly elevated even after atipamezole. In contrast, no significant hemodynamic changes were detected with Med-Vat regardless of atipamezole (Figures 1 and 2). In study IV, except for MAP and SVR, both of which significantly decreased from baseline, hemodynamic variables did not significantly change from pre-treatment values regardless of the presence of vatinoxan (Table 6). Oxygen delivery decreased significantly after all treatments and remained below the pre-treatment level until atipamezole (study I). Similarly, arterial oxygen content (CaO_2) decreased from baseline, but was significantly higher with medetomidine-ketamine than treatments that included vatinoxan (study I). In study II, the changes in DO_2, and CaO_2 were comparable with those in study I, where atipamezole significantly increased both values.

Initially, PaO_2 decreased significantly after all treatments in studies I and II (Tables 4 and 5). In study IV, pre-administration of vatinoxan prevented the increase in alveolar to arterial difference in partial pressure of oxygen ($P_{(A-a)}O_2$) and the decreases in PaO_2, PvO_2, and PaO_2/FiO_2 ratio (Table 6). Moreover, vatinoxan prevented both the decrease in C_{dyn} and the need to increase PIP (Figure 3). On CT imaging, lung density was significantly increased after dexmedetomidine alone, while pre-treatment with vatinoxan prevented those changes (Figure 4). For the bronchoscopy, no excessive mucus or edema were detected within the bronchi after either treatment. Additionally, no visual abnormalities were observed in BAL fluid and there were no significant differences in differential white blood cell counts between treatments.

5.2 Plasma concentrations of drugs

Dexmedetomidine, levomedetomidine, and vatinoxan concentrations in plasma from studies I and II are presented in Figures 5 and 6. The concentrations of the enantiomers did not differ from each other in any of the studies. Additionally, in study I, no significant differences in their concentrations in plasma were detected between treatments (Figure 5). Conversely, in study II, the dexmedetomidine and levomedetomidine concentrations were approximately three times higher with the inclusion of vatinoxan at 20 minutes (Figure 6). Likewise, concentrations of both enantiomers were approximately doubled at 30 minutes with the addition of vatinoxan (study III). After atipamezole, the concentrations of dexmedetomidine and levomedetomidine in plasma were significantly higher when medetomidine was administered alone (Figures 5 and 6). Pre-administered intravenous vatinoxan did not affect the exposure to dexmedetomidine in sevoflurane-anesthetized sheep (study IV). Vatinoxan concentrations in study II did not differ significantly between treatments (Figure 7). No significant

changes were detected in atipamezole concentrations between treatments, regardless of the presence or absence of either vatinoxan or ketamine. For example, 30 minutes after reversal (T90, study I; T60, study II) the concentrations of atipamezole were (range) 31.7–36.4 ng/mL (study I) and 34.7–35.6 ng/mL (study II). Similarly, the concentrations of ketamine and norketamine did not significantly differ between treatments (study I; Figure 8).

5.3 Sedative effects

In study I, sedation scores did not significantly differ between treatments before or after reversal with atipamezole (Table 7). Areas under the time-sedation curves ($AUC_{sed0–70}$) were [median (range)] 540 (449–578), 473 (374–529), 482 (406–795), and 497 (426–637) for Med-Ket, Med-Ket-Vat150, Med-Ket-Vat300, and Med-Ket-Vat600; respectively. Addition of vatinoxan significantly intensified the initial sedative effect of medetomidine (studies II and III). After reversal, the scores were significantly lower with treatments that included vatinoxan when compared to medetomidine alone (Table 8, Figure 9). In study I, immediately after atipamezole administration, some animals exhibited signs of agitation accompanied with tremor of the fascial muscles. No such signs were observed in studies II and III.

5.4 Rectal temperature

Rectal temperatures declined significantly from 30 minutes onwards after all treatments in study I, including the period after atipamezole, but no clinically relevant hypothermia (i.e. temperature < 36 °C) was observed (Table 4). In study II, a significant decrease was recorded after 40 minutes, but only for treatments that did not include atipamezole (Table 5). No changes in rectal temperature were detected in study IV (Table 6).

5.5 Hemoglobin content

In general, the Hb concentration decreased after all treatments, although the decline was more pronounced after treatments that included vatinoxan. Reversal with atipamezole restored the Hb values (studies I and II; Tables 4 and 5).

5.6 Plasma glucose

Medetomidine significantly increased plasma glucose concentrations, which remained elevated even after atipamezole. Both concurrent (studies I and II) and pre-administration of vatinoxan (study IV), prevented these increases (Tables 4–6).

Table 4. Mean ± SD of CO, SAP, DAP, PaO_2, $PaCO_2$, Hb, glucose, DO_2, SaO_2, CaO_2, and rectal temperature (Temp.) from study I (Adam et al., 2018a) in nine sheep sedated with medetomidine-ketamine with and without vatinoxan and administered atipamezole 60 minutes later to reverse sedation (See Table 2 for treatments key). *Statistically significantly different ($P < 0.05$) from baseline within the same treatment (row). †Significant difference ($P < 0.05$) from Med-Ket within the same column.

		Time after administration (min)				
Variable	Treatment	Baseline	15	30	45	90
CO (L/min)	Med-Ket	4.2 ± 0.54	3.4 ± 1.26*	3.6 ± 0.54	3.8 ± 0.96	4.5 ± 0.81
	Med-Ket-Vat150	4.7 ± 0.67	3.9 ± 0.70*	4.6 ± 0.87	4.6 ± 1.13	5.2 ± 1.77
	Med-Ket-Vat300	4.9 ± 0.90	4.6 ± 1.59	4.2 ± 0.56	4.1 ± 0.57	4.9 ± 1.28
	Med-Ket-Vat600	4.9 ± 1.51	4.1 ± 1.09*	4.0 ± 1.30*	3.7 ± 0.93*	5.8 ± 2.51
SAP (mmHg)	Med-Ket	120 ± 8	136 ± 10*	132 ± 8*	124 ± 19†	130 ± 17
	Med-Ket-Vat150	125 ± 15	127 ± 13†	114 ± 11†	99 ± 7*†	129 ± 12
	Med-Ket-Vat300	123 ± 11	122 ± 9†	102 ± 15*†	91 ± 14*†	133 ± 8*
	Med-Ket-Vat600	123 ± 14	109 ± 17*†	89 ± 12*†	84 ± 9*†	131 ± 7
DAP (mmHg)	Med-Ket	91 ± 4	103 ± 10*	102 ± 12*	97 ± 16	95 ± 7
	Med-Ket-Vat150	95 ± 11	98 ± 7	88 ± 9†	77 ± 8*†	94 ± 13
	Med-Ket-Vat300	93 ± 10	94 ± 6†	78 ± 9*†	68 ± 13*†	98 ± 9
	Med-Ket-Vat600	92 ± 9	85 ± 13†	68 ± 13*†	64 ± 11*†	99 ± 7*
PaO_2 (mmHg)	Med-Ket	98.1 ± 7.8	71.1 ± 12.4*	68.6 ± 9.3*	73.7 ± 10.0*	99.5 ± 12.7
	Med-Ket-Vat150	99.0 ± 7.7	76.3 ± 13.7*	81.9 ± 11.3*†	86.2 ± 8.6*†	103.0 ± 6.3
	Med-Ket-Vat300	103.3 ± 6.6	79.2 ± 11.8*†	83.1 ± 7.9*†	92.9 ± 8.0*†	97.3 ± 9.4
	Med-Ket-Vat600	94.7 ± 5.7	79.7 ± 11.3*†	81.7 ± 8.2*†	89.6 ± 10.9†	92.8 ± 7.8
$PaCO_2$ (mmHg)	Med-Ket	39.5 ± 4.6	49.5 ± 8.6*	51.5 ± 6.2*	49.4 ± 6.9*	41.0 ± 7.9
	Med-Ket-Vat150	40.2 ± 2.3	52.2 ± 7.6*	51.9 ± 4.9*	50.0 ± 4.4*	43.2 ± 3.7
	Med-Ket-Vat300	39.8 ± 3.3	48.9 ± 7.3*	51.4 ± 7.3*	46.8 ± 3.4*	42.4 ± 2.2
	Med-Ket-Vat600	39.6 ± 2.8	50.8 ± 5.2*	51.2 ± 4.0*	45.7 ± 6.2*	41.3 ± 6.4
Glucose (mmol/L)	Med-Ket	4.6 ± 1.5	5.8 ± 1.5*	6.7 ± 1.8*	7.8 ± 1.9*	8.6 ± 2.5*
	Med-Ket-Vat150	4.3 ± 0.5	4.5 ± 0.5†	4.7 ± 0.9†	4.7 ± 0.9†	5.0 ± 0.8*†
	Med-Ket-Vat300	4.9 ± 1.8	5.0 ± 1.9†	4.9 ± 2.0†	4.9 ± 1.9†	5.1 ± 1.9†
	Med-Ket-Vat600	4.3 ± 0.3	4.3 ± 0.6†	4.2 ± 0.6†	4.1 ± 0.5†	4.1 ± 0.5†
Hb (g/dL)	Med-Ket	9.4 ± 0.8	8.88 ± 0.86*	8.53 ± 0.59*	8.58 ± 0.95*	9.58 ± 1.12
	Med-Ket-Vat150	9.3± 0.97	8.1 ± 0.78*†	7.7 ± 0.60*†	7.5 ± 0.60*†	9.2 ± 0.93
	Med-Ket-Vat300	9.1 ± 0.61	8.2 ± 0.83*†	7.7 ± 0.63*†	7.5 ± 0.64*†	9.2 ± 0.93
	Med-Ket-Vat600	9.4 ± 1.05	8.3 ± 0.77*†	7.7 ± 0.67*†	7.5 ± 0.63*†	8.9 ± 0.81
SaO_2 (%)	Med-Ket	95.4 ± 0.9	87.4 ± 6.3*	86.8 ± 5.1*	89.1 ± 4.1*	95.3 ± 1.8
	Med-Ket-Vat150	95.5 ± 1.0	89.6 ± 5.6*	91.8 ± 3.2*†	93.2 ± 1.7†	96.0 ± 0.6
	Med-Ket-Vat300	96.0 ± 0.7	90.9 ± 3.4*	92.4 ± 2.2*†	94.5 ± 1.2†	95.0 ± 1.7
	Med-Ket-Vat600	94.9 ± 0.8	91.1 ± 3.6*†	92.0 ± 2.2*†	93.7 ± 2.2†	94.5 ± 1.4
DO_2 (mL/kg/min)	Med-Ket	12.2 ± 2.1	8.9 ± 2.2*	9.3 ± 1.4*	8.7 ± 1.3*	13.9 ± 3.4
	Med-Ket-Vat150	12.9 ± 1.6	8.5 ± 1.0*	10.2 ± 2.1*	10.1 ± 2.4*	14.0 ± 3.0
	Med-Ket-Vat300	14.2 ± 3.4	10.9 ± 3.6*	9.9 ± 2.3*	9.6 ± 1.8*	14.0 ± 3.8
	Med-Ket-Vat600	14.4 ± 6.9	9.9 ± 2.9*	9.3 ± 3.1*	8.2 ± 2.0*	15.0 ± 3.0
CaO_2 (mL/dL)	Med-Ket	128.5 ± 10.6	114.3 ± 13.0*	106.5 ± 7.1*	111.1 ± 10.9*	130.9 ± 16.1
	Med-Ket-Vat150	127.7 ± 12.1	105.3 ± 9.9*†	102.2 ± 6.2*†	101.3 ± 7.3*†	127.3 ± 16.9
	Med-Ket-Vat300	126.2 ± 8.4	108.1 ± 12.3*	103.2 ± 8.2*	102.8 ± 8.7*†	123.5 ± 13.5
	Med-Ket-Vat600	128.0 ± 13.6	109.1 ± 9.6*	101.5 ± 7.9*†	100.5 ± 8.2*†	117.5 ± 9.6
Temp (°C)	Med-Ket	39.4 ± 0.49	39.3 ± 0.51	38.9 ± 0.58*	38.8 ± 0.68*	38.6 ± 0.86*
	Med-Ket-Vat150	39.3 ± 0.37	39.3 ± 0.25	39.0 ± 0.36	38.7 ± 0.29*	38.7 ± 0.36*
	Med-Ket-Vat300	39.4 ± 0.35	39.2 ± 0.49	39.0 ± 0.52*	38.6 ± 0.53*	38.6 ± 0.72*
	Med-Ket-Vat600	39.1 ± 0.62	39.2 ± 0.42	38.8 ± 0.51*	38.5 ± 0.57*	38.6 ± 0.58*

Table 5. Mean ± SD of CO, SAP, DAP, PaO_2, $PaCO_2$, Hb, glucose, DO_2, SaO_2, CaO_2, and rectal temperature (Temp.) from study II (Adam et al., 2018b) in eight sheep sedated with medetomidine with and without vatinoxan and administered atipamezole or saline 30 minutes to reverse sedation (See Table 2 for treatments key). *Significant difference from the baseline within the same treatment, †Significant difference between MED and Med-Vat, ‡Significant difference between MED+ATI and Med-Vat+ATI, §Significant difference between MED and MED+ATI, and #Significant difference between Med-Vat and Med-Vat+ATI ($P < 0.05$).

		Time after administration (min)					
Variable	Treatment	Baseline	20	40	60	75	90
CO (L/min)	MED	4.47 ± 0.81	3.08 ± 0.32	3.32 ± 0.81	3.49 ± 0.69	3.18 ± 0.41	3.14 ± 0.61
	MED+ATI	4.51 ± 0.71	3.31 ± 0.55	3.83 ± 0.85	4.14 ± 1.06	4.27 ± 0.81	4.29 ± 1.09
	Med-Vat	4.68 ± 0.92	3.82 ± 0.60	4.07 ± 0.76	3.55 ± 0.44	3.79 ± 0.77	4.01 ± 1.10
	Med-Vat+ATI	4.29 ± 1.25	4.19 ± 0.67	4.49 ± 1.32	4.90 ± 1.54	4.50 ± 1.55	4.87 ± 1.60
SAP (mmHg)	MED	126 ± 11	122 ± 9	120 ± 13†	114 ± 12†	110 ± 12	108 ± 13
	MED+ATI	118 ± 8	117 ± 12	130 ± 16	125 ± 11§	130 ± 19§	124 ± 10§
	Med-Vat	121 ± 9	117 ± 9	100 ± 12*	100 ± 9*	103 ± 9*	111 ± 12
	Med-Vat+ATI	118 ± 14	119 ± 11	131 ± 19#	128 ± 13#	126 ± 15#	126 ± 14
DAP (mmHg)	MED	97 ± 9	95 ± 8	94 ± 8†	91 ± 8†	89 ± 9	88 ± 10
	MED+ATI	91 ± 6	91 ± 8	102 ± 16	96 ± 9	100 ± 17	94 ± 8
	Med-Vat	94 ± 6	92 ± 6	80 ± 8*	77 ± 5*	79 ± 6*	82 ± 6*
	Med-Vat+ATI	91 ± 12	94 ± 7	99 ± 16#	96 ± 11#	96 ± 10#	95 ± 8#
PaO_2 (mmHg)	MED	102 ± 7.3	90 ± 5.7*	89 ± 3.5*	89 ± 7.9*	84 ± 5.1*	88 ± 6.1*
	MED+ATI	104 ± 4.5	89 ± 8.4*	90 ± 11.4*	99 ± 12.2	102 ± 9.9§	102 ± 5.6§
	Med-Vat	96 ± 10.1	87 ± 14	85 ± 10.6	89 ± 16	92 ± 7.9	91 ± 7.4
	Med-Vat+ATI	101 ± 4.3	90 ± 11.4*	96 ± 9.7	100 ± 6.6	99 ± 8.2	106 ± 9.8#
$PaCO_2$ (mmHg)	MED	38.3 ± 2.9	43.3 ± 4.1	44.7 ± 3.2*	45.4 ± 1.9*	46.7 ± 4.4*	44.9 ± 3.5*
	MED+ATI	38.6 ± 2.6	45.2 ± 3.0*	41.8 ± 2.8*	41.9 ± 2.9§	41.3 ± 2.6§	42.1 ± 2.7
	Med-Vat	40.1 ± 2.1	47.0 ± 2.4*	46.0 ± 2.1*	45.4 ± 2.9*	44.7 ± 3.9	45.1 ± 3.4*
	Med-Vat+ATI	39.3 ± 3.1	47.5 ± 2.9*	44.9 ± 8.6	41.0 ± 1.3#	41.5 ± 1.7	40.9 ± 4.2
Glucose (mmol/L)	MED	3.9 ± 0.4	5.9 ± 1.1*†	7.2 ± 1.5*†	8.2 ± 1.9*†	8.8 ± 2.1*†	9.3 ± 2.4*†
	MED+ATI	3.8 ± 0.4	6.1 ± 0.6*‡	6.9 ± 1.0*‡	6.3 ± 1.1*‡§	6.3 ± 1.1*‡§	6.1 ± 1.2*§
	Med-Vat	3.9 ± 0.4	4.4 ± 0.6	4.3 ± 0.7	4.1 ± 0.7	4.2 ± 0.8	4.2 ± 0.8
	Med-Vat+ATI	4.0 ± 0.3	4.6 ± 0.8	4.5 ± 0.9	4.6 ± 0.9	4.6 ± 1.0	4.7 ± 1.1
Hb (g/dL)	MED	8.79 ± 0.89	7.78 ± 0.92	7.31 ± 0.55*	7.20 ± 0.50*	7.28 ± 0.56*	7.35 ± 0.55*
	MED+ATI	8.26 ± 0.98	7.26 ± 0.58	8.26 ±1.08	8.04 ± 1.00	8.49 ± 1.43	8.66 ± 2.09
	Med-Vat	8.46 ± 0.97	7.56 ± 0.52	7.07 ± 0.52*	6.99 ± 0.56*	6.98 ± 0.47*	7.04 ± 0.45*
	Med-Vat+ATI	8.36 ± 0.65	7.45 ± 0.57	8.34 ± 0.76#	9.27 ± 1.56#	9.34 ± 1.48#	9.09 ± 1.56#
SaO_2 (%)	MED	95.8 ± 0.8	94.1 ± 1.2*	93.9 ± 0.6*	93.8 ± 1.6	92.8 ± 1.3*	93.6 ± 1.1*
	MED+ATI	96.1 ± 0.4	93.7 ± 2.0*	93.6 ± 2.4	95.2 ± 1.7	95.7 ± 1.4§	95.9 ± 0.6§
	Med-Vat	94.9 ± 2.0	92.9 ± 3.1	92.6 ± 3.0	93.0 ± 3.6	94.3 ± 1.7	94.1 ± 1.5
	Med-Vat+ATI	95.8 ± 0.5	93.6 ± 2.4	95.0 ± 1.4	95.6 ± 0.7	95.5 ± 0.8	96.3 ± 0.9#
DO_2 (mL/kg/min)	MED	11.1 ± 3.6	6.7 ± 0.8*	6.4 ± 0.5*	6.7 ± 0.7*	6.2 ± 0.8*	6.2 ± 0.9*
	MED+ATI	10.1 ± 1.8	6.5 ± 1.7*	8.7 ± 3.0§	9.0 ± 3.1	9.9 ± 3.0§	10.0 ± 2.9§
	Med-Vat	10.4 ± 1.4	7.8 ± 2.1	7.4 ± 1.5*	6.5 ± 0.9*	7.0 ± 1.3*	7.5 ± 1.9*
	Med-Vat+ATI	9.4 ± 1.0	8.2 ± 1.6	10.5 ± 2.3#	11.4 ± 2.4#	10.9 ± 2.8#	11.7 ± 3.3#
CaO_2 (mL/L)	MED	12.0 ± 1.3	10.4 ± 1.2	9.8 ± 0.7*	9.7 ± 0.8*	9.7 ± 0.7*	9.8 ± 0.7*
	MED+ATI	11.4 ± 1.3	9.7 ± 0.7*	10.8 ± 1.6	10.9 ± 1.2§	11.6 ± 1.8§	11.9 ± 2.8
	Med-Vat	11.4 ± 1.0	9.9 ± 0.8*	9.3 ± 0.7*	9.3 ± 0.8*	9.4 ± 0.6*	9.5 ± 0.6*
	Med-Vat+ATI	11.5 ± 0.8	10.0 ± 0.7*	11.5 ± 0.9#	12.4 ± 2.1#	12.7 ± 2.0#	12.5 ± 2.1#
Temp (°C)	MED	39.3 ± 0.22	39.3 ± 0.17	38.9 ± 0.45	38.7 ± 0.55	38.4 ± 0.60*	38.3 ± 0.75*
	MED+ATI	39.4 ± 0.23	39.4 ± 0.29	39.3 ± 0.47	39.2 ± 0.48	39.1 ± 0.45	39.1 ± 0.37§
	Med-Vat	39.3 ± 0.24	39.2 ± 0.17	38.7 ± 0.32	38.4 ± 0.47*	38.3 ± 0.52*	38.4 ± 0.57*
	Med-Vat+ATI	39.3 ± 0.33	39.4 ± 0.38	38.9 ± 0.52	39.0 ± 0.57	38.9 ± 0.65	39.0 ± 0.66

Table 6. Mean ± SD of HR, SAP, DAP, MAP, SPAP, DPAP, MPAP, CVP, CO, SVR, PaO_2, $PaCO_2$, PvO_2, $PvCO_2$, PaO_2/FiO_2, Hb, glucose, and rectal temperature from study IV (Adam et al., 2018c) in seven sevoflurane-anesthetized sheep pretreated with vatinoxan or saline and 10 minutes later administered dexmedetomidine (See Table 2 for treatments key). *Significant difference from the baseline within the same treatment, †Significant difference between treatments ($P < 0.05$).

		Time-points				
Variable	Treatment	Baseline	T-7	T5	T15	T25
CO (L/min)	DEX	2.87 ± 0.78	3.15 ± 0.72	2.82 ± 0.52	3.05 ± 0.31	3.37 ± 0.25
	VAT+DEX	2.77 ± 0.26	3.63 ± 0.69*	3.25 ± 0.68	3.04 ± 0.41	3.15 ± 0.44
HR (bpm)	DEX	84 ± 16	84 ± 16	74 ± 9	75 ± 11	76 ± 11
	VAT+DEX	82 ± 8	85 ± 6	76 ± 9	73 ± 8	73 ± 9
SAP (mmHg)	DEX	102 ± 17	105 ± 14	109 ± 9	106 ± 11	92 ± 6
	VAT+DEX	105 ± 9	101 ± 8	106 ± 6	86 ± 5	80 ± 5
DAP (mmHg)	DEX	84 ± 18	87 ± 13	93 ± 7	89 ± 9	72 ± 7
	VAT+DEX	85 ± 12	81 ± 8	88 ± 4	66 ± 3	60 ± 422
MAP (mmHg)	DEX	92 ± 17	95 ± 13	100 ± 8	97 ± 10†	81 ± 6†
	VAT+DEX	94 ± 10	90 ± 8	96 ± 4	75 ± 4*	69 ± 5*
SPAP (mmHg)	DEX	22 ± 3	23 ± 3	25 ± 4	23 ± 4	22 ± 7
	VAT+DEX	24 ± 4	23 ± 4	23 ± 4	24 ± 6	22 ± 4
DPAP (mmHg)	DEX	15 ± 3	15 ± 4	18 ± 4	17 ± 4	15 ± 6
	VAT+DEX	17 ± 2	15 ± 1	15 ± 1	16 ± 5	14 ± 2
MPAP (mmHg)	DEX	18 ± 3	18 ± 3	21 ± 4	20 ± 3	18 ± 5
	VAT+DEX	20 ± 2	19 ± 3	19 ± 2	20 ± 5	17 ± 2
CVP (mmHg)	DEX	10 ± 3	9 ± 2	13 ± 2	11 ± 2	10 ± 3
	VAT+DEX	13 ± 4	11 ± 3	12 ± 4	11± 3	11 ± 3
SVR (dynes/sec/cm^5)	DEX	2328 ± 440	2279 ± 260†	2547 ± 550	2256 ± 262†	1676 ± 203*
	VAT+DEX	2363 ± 442	1784 ± 306*	2121 ± 396	1678 ± 180*	1480 ± 119*
PaO_2 (mmHg)	DEX	289 ± 23	280 ± 30	195 ± 85*	213 ± 78*	259 ± 37
	VAT+DEX	280 ± 36	288 ± 23	289 ± 23†	289 ± 19†	291 ± 16†
PvO_2 (mmHg)	DEX	43 ± 11	42 ± 10	35 ± 7*	37 ± 5*	39 ± 5
	VAT+DEX	41 ± 7	48 ± 9	41 ± 7†	42 ± 7†	42 ± 7
$PaCO_2$ (mmHg)	DEX	37 ± 2	37 ± 1	44 ± 8*	43 ± 5*	41 ± 3
	VAT+DEX	36 ± 3	37 ± 1	37 ± 2†	38 ± 2†	37 ± 1
$PvCO_2$ (mmHg)	DEX	45 ± 4	45 ± 2	48 ± 5	50 ± 4*	50 ± 3*
	VAT+DEX	43 ± 5	43 ± 2	44 ± 2	45 ± 5†	45 ± 2†
PaO_2/FiO_2	DEX	577 ± 46	561 ± 60	392 ± 171*	426 ± 157*	518 ± 74*
	VAT+DEX	560 ± 72	575 ± 46	578 ± 46†	578 ± 38†	582 ± 32†
$P_{(A-a)}O_2$ (mmHg)	DEX	31 ± 17	34 ± 30	110 ± 77*	95 ± 74*	51 ± 35
	VAT+DEX	41 ± 32	27 ± 22	25 ± 22†	25 ± 18†	23 ± 16†
Hb (g/dL)	DEX	7.7 ± 0.8	7.4 ± 0.8	7.5 ± 0.8	7.6 ± 0.8	7.5 ± 0.8
	VAT+DEX	7.9 ± 0.7	7.8 ± 0.9	7.6 ± 0.8	7.5 ± 0.8	7.3 ± 0.8
Glucose (mmol/L)	DEX	3.6 ± 0.3	3.5 ± 0.3	3.6 ± 0.3	5.8 ± 1.1	7.0 ± 1.5
	VAT+DEX	4.0 ± 0.5	3.8 ± 0.3	3.7 ± 0.4	3.7 ± 0.3	3.7 ± 0.4
Temp (°C)	DEX	38.5 ± 0.52	38.3 ± 0.39	38.1 ± 0.31	38.3 ± 0.34	38.3 ± 0.50
	VAT+DEX	38.2 ± 0.24	38.2 ± 0.22	38.1 ± 0.26	38.2 ± 0.30	38.1 ± 0.31

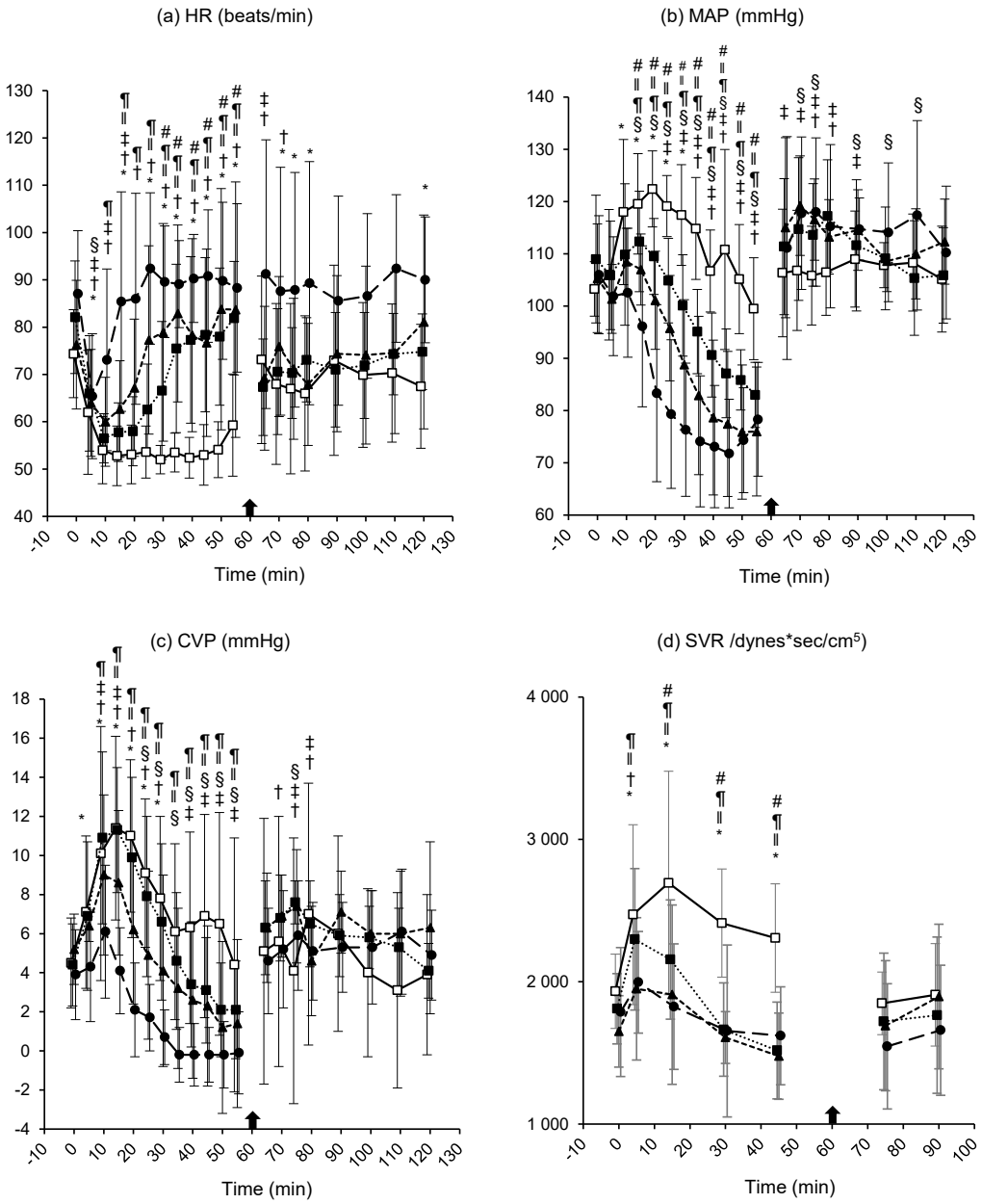

Figure 1. Mean ± SD of (a) heart rate (HR), (b) mean arterial pressure (MAP), (c) central venous pressure (CVP), and (d) systemic vascular resistance (SVR) in sheep treated with IM medetomidine and ketamine (Med-Ket [white squares]) with and without three different doses of vatinoxan at 150 µg/kg (Med-Ket-Vat150 [black squares]), 300 µg/kg (Med-Ket-Vat300 [triangles]), and 600 µg/kg (Med-Ket-Vat600 [circles]), and received IM atipamezole 60 minutes later (arrow). *, †, ‡, §Within a treatment (*Med-ket, † Med-Ket-Vat150, ‡ Med-Ket-Vat300, and § Med-Ket-Vat600), the value differs significantly ($P < 0.05$) from the baseline value. ‖, ¶, # Within a time point, value for a treatment that included vatinoxan (‖ Med-Ket-Vat300, ¶ Med-Ket-Vat600, and # Med-Ket-Vat150) differs significantly ($P < 0.05$) from the value for the Med-Ket treatment.

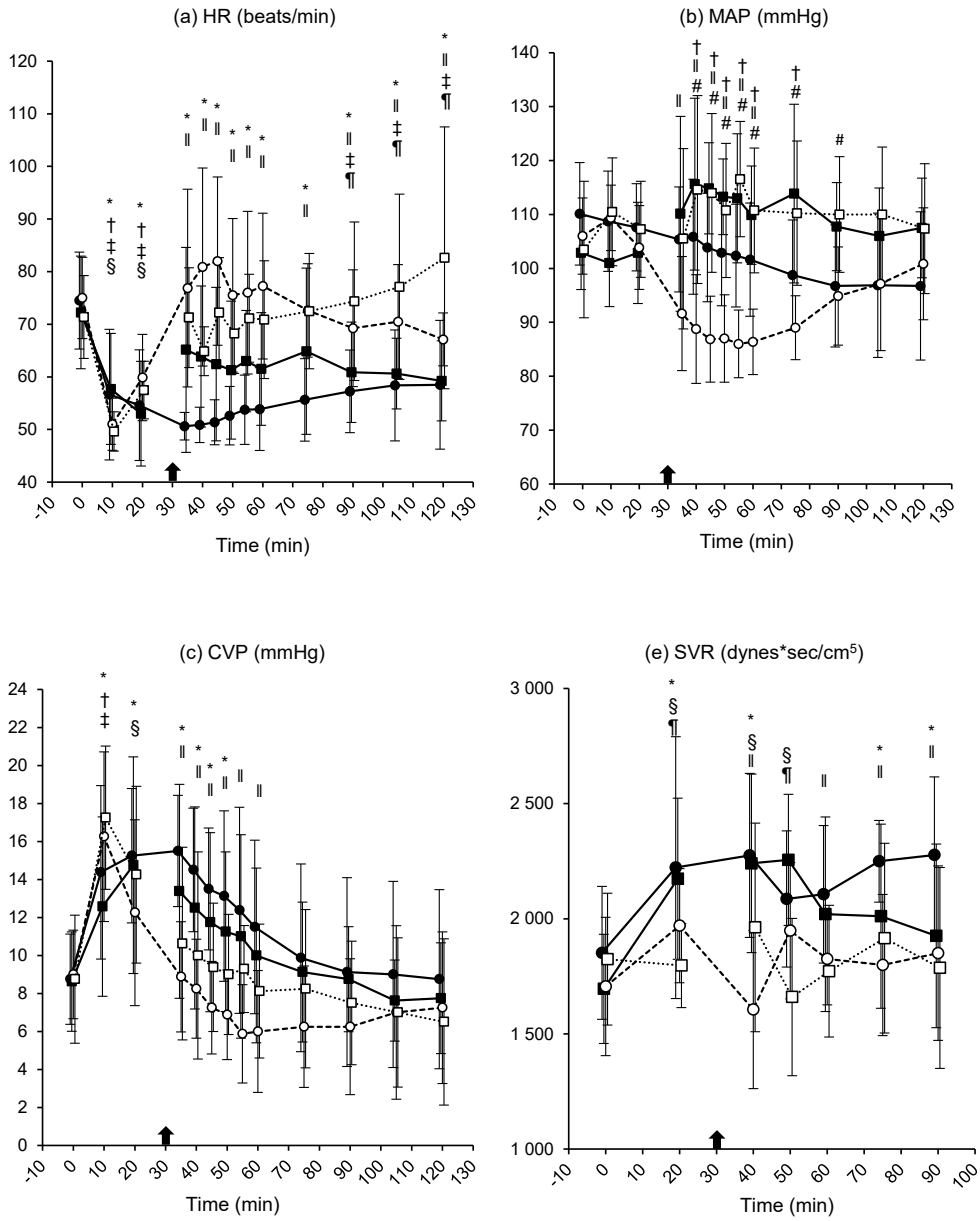

Figure 2. Mean ± SD of (a) heart rate (HR), (b) mean arterial pressure (MAP), (c) central venous pressure (CVP), (d) and systemic vascular resistance (SVR) in sheep treated with IM medetomidine alone (MED [black circles]) or combined with vatinoxan (Med-Vat [white circles]), and received either atipamezole or equal volume of saline (MED+ATI, Med-Vat+ATI [black and white squares respectively]) 30 minutes later (arrow). *†‡§Significant difference from the baseline within the same treatment (MED, Med-Vat, MED+ATI, and, Med-Vat+ATI), ‖significant difference between MED and Med-Vat, ¶Significant differences between MED+ATI and Med-Vat+ATI, |significant difference between MED and MED+ATI, and #significant difference between Med-Vat and Med-Vat+ATI; ($P < 0.05$).

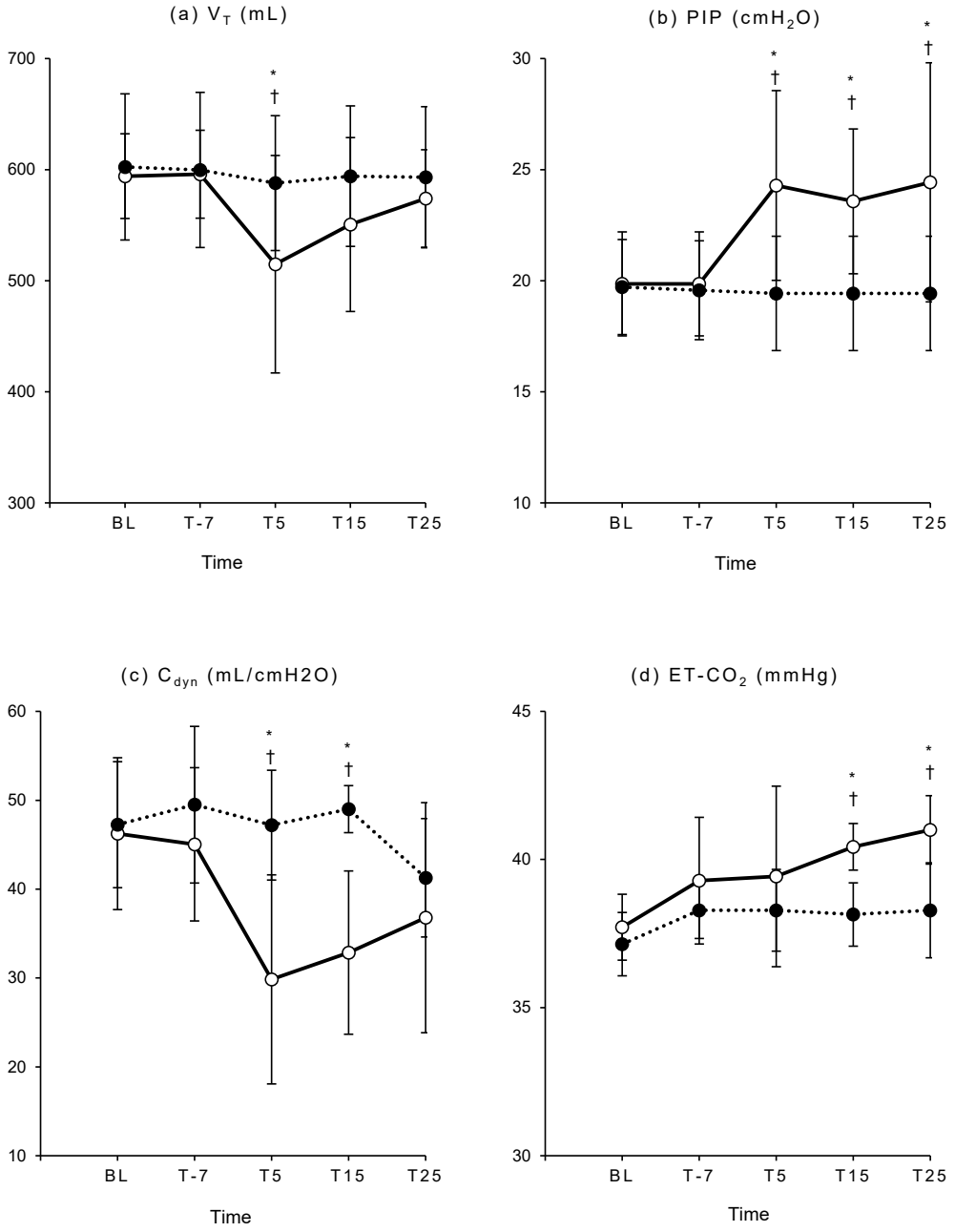

Figure 3. Mean ± SD of (a) tidal volume (V_T), (b) peak inspiratory pressure (PIP), (c) dynamic compliance (C_{dyn}), and (d) end-tidal carbon dioxide (ET-CO2) in sevoflurane-anesthetized sheep that were pre-treated with vatinoxan (VAT+DEX [black circles]) or saline and 10 minutes later received dexmedetomidine (DEX [white circles]). A positive end-expiratory pressure (PEEP) of 5 mmHg was applied throughout the study. †Significant difference between treatments, *significantly different from BL ($P < 0.05$).

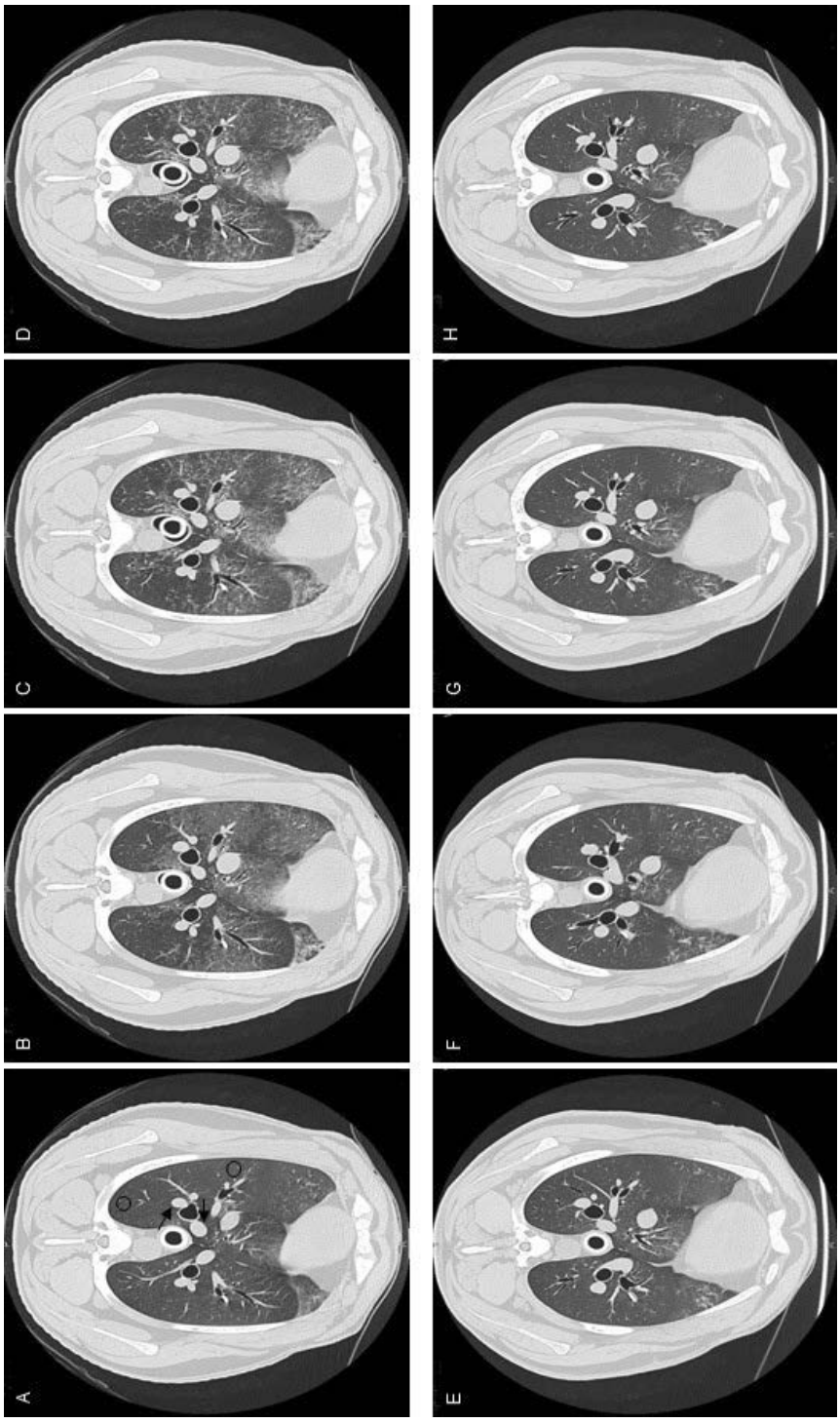

Figure 4. Transverse CT images at the level of the mid-caudal lung lobes in a sheep that was pre-treated with vatinoxan (VAT+DEX) or saline and 10 minutes later received dexmedetomidine (DEX) at baseline, T10, T20, and T30 (A–D; DEX); and at T-2, T10, T20, and T30 (E–H; VAT+DEX). Note in image A: arrows indicate the caudal pulmonary vein and artery, whereas the circles indicate the regions of interest (ROI).

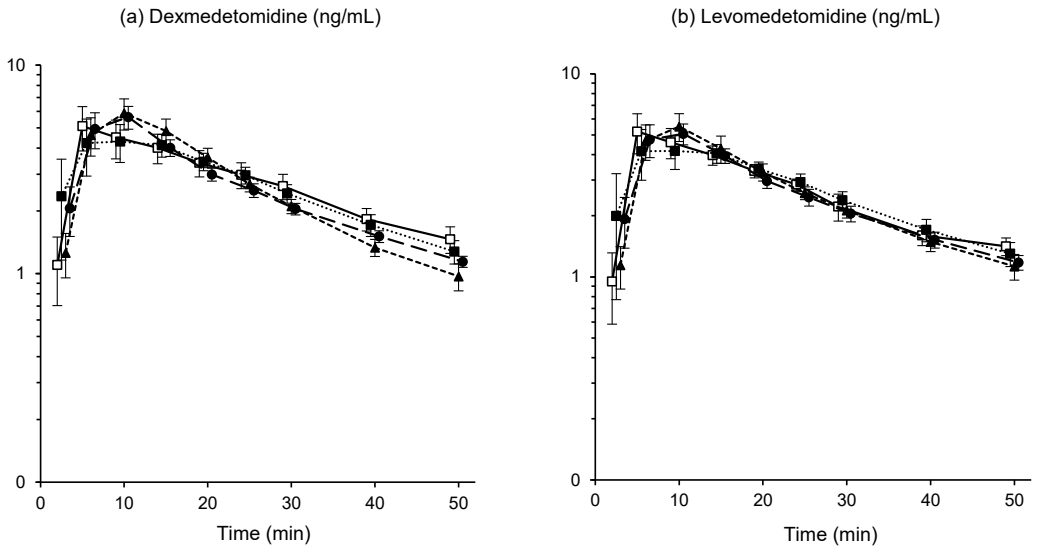

Figure 5. Mean ± SE plasma concentration of (a) dexmedetomidine and (b) levomedetomidine in sheep treated with IM medetomidine and ketamine (Med-Ket [white squares]) with and without three different doses of vatinoxan (Med-Ket-Vat150, Med-Ket-Vat300 & Med-Ket-Vat600 [black squares, triangles & circles, respectively]), and received IM atipamezole 60 minutes later.

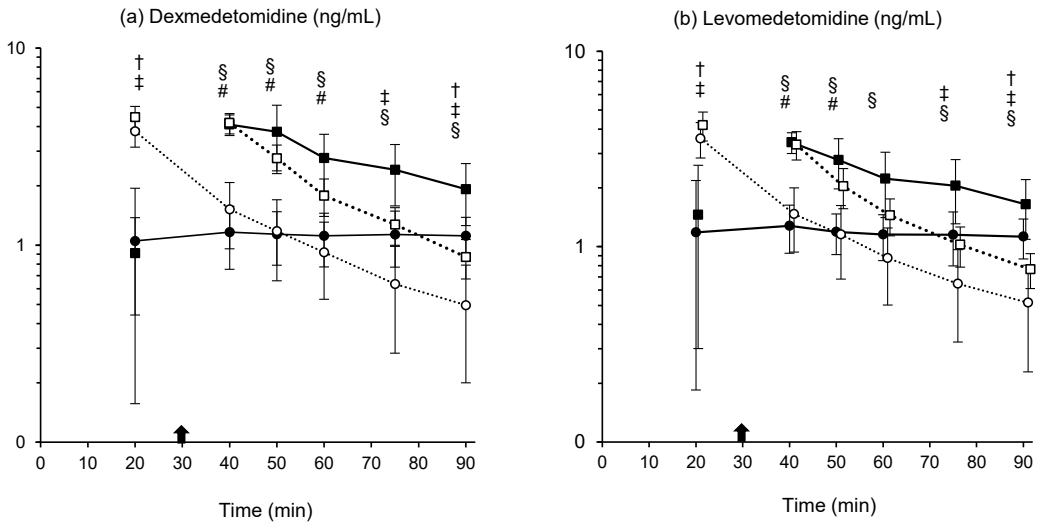

Figure 6. Mean ± SD of (a) dexmedetomidine and (b) levomedetomidine in sheep treated with IM medetomidine alone (MED [black circles]) or combined with vatinoxan (Med-Vat [white circles]), followed by IM atipamezole (MED+ATI and Med-Vat+ATI [black and white squares respectively]) or an equal volume of saline 30 minutes later (arrow). †Significant difference between MED and MMK, ‡Significant difference between MED+ATI and Med-Vat+ATI, §Significant difference between MED and MED+ATI, and #Significant difference between MMK and Med-Vat+ATI ($P < 0.05$).

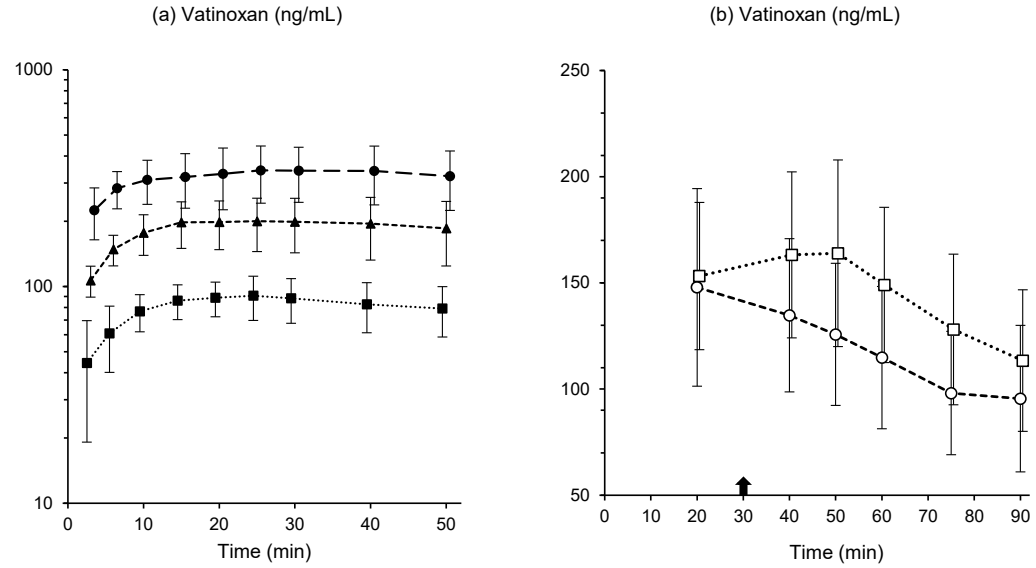

Figure 7. Mean ± SD of vatinoxan in sheep (a) treated with IM medetomidine (30 µg/kg) and ketamine (1 mg/kg) combined with three different doses of vatinoxan (150, 300 & 600 µg/kg; Med-Ket-Vat150, Med-Ket-Vat300 & Med-Ket-Vat600 [black squares, triangles & circles], respectively), and (b) treated with IM medetomidine (30 µg/kg) combined with vatinoxan 300 µg/kg (Med-Vat [white circles]), and followed by IM atipamezole 150 µg/kg (Med-Vat+ATI [white squares]) or saline 30 minutes later (arrow).

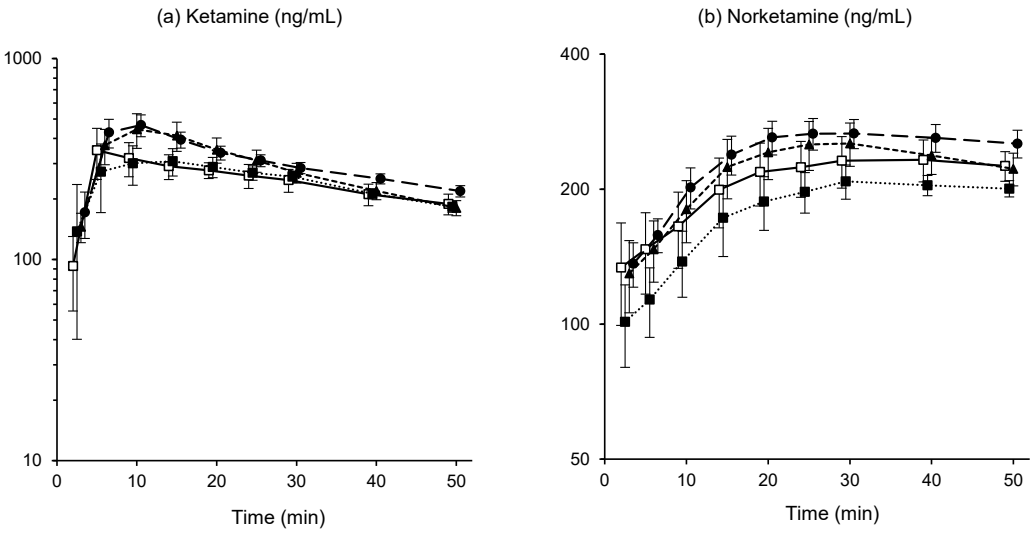

Figure 8. Mean ± SE plasma concentration of (a) ketamine and (b) norketamine in sheep treated with IM medetomidine and ketamine (Med-Ket) with and without three different doses of vatinoxan (Med-Ket-Vat150, Med-Ket-Vat300 & Med-Ket-Vat600 [black squares, triangles & circles, respectively]), and received IM atipamezole 60 minutes later.

Table 7. Median (range) of visual analogue sedation scores in nine sheep sedated with with IM medetomidine and ketamine (Med-Ket) alone and combined in the same syringe with three different doses of vatinoxan (Med-Ket-Vat150, Med-Ket-Vat300 & Med-Ket-Vat600), and received IM atipamezole 60 minutes later to reverse sedation (See Table 2 for treatments key and doses).

	Time (min)							
Treatment	Baseline	5	15	30	45	75	90	120
Med-Ket	0 (0– 0)	3.3 (0.5–6)	8 (6–9)	9.5 (9–10)	9 (8–9)	0 (0–2)	0 (0–1)	0 (0– 4)
Med-Ket-Vat150	0 (0–0)	4.3 (1– 5)	7 (4–9)	9 (7–10)	9 (7– 9)	0 (0–1)	0 (0–0)	0 (0–1)
Med-Ket-Vat300	0 (0–0)	3.8 (3– 7)	8.5 (7–10)	9.5 (9–10)	9 (8–9)	0 (0–0)	0 (0–0)	0 (0–0)
Med-Ket-Vat600	0 (0–0)	3.4 (1–6)	8.3 (5– 10)	9.7 (9– 10)	9 (8–9)	0 (0–0)	0 (0–0)	0 (0–0)

Table 8. Median (range) of visual analogue sedation scores in eight sheep sedated with IM medetomidine (MED) alone and combined in the same syringe with vatinoxan (Med-Vat), and administered IM atipamezole (MED+ATI, Med-Vat+ATI) or equal volume of saline at 30 minutes to reverse sedation (See Table 2 for treatments key and doses). Significant difference from baseline is indicated by *; while † and ‡ denote significant differences between MED and Med-Vat, and MED+ATI and Med-Vat+ATI, respectively.

	Time (min)							
Treatment	Baseline	10	20	40	60	75	90	120
MED	0 (0– 0)	4 (2–6)*†	7 (3–8)*†	6 (4–8)*	5.5 (4–7)*	5.5 (3–7)*	5.5 (3–6)*	3.5 (2– 4)*†
MED+ATI	0 (0 –0)	4.5 (2– 7)*‡	7 (4–9)*‡	2.5 (0–7)*	0.5 (0– 7)*‡	0.5 (0–5)	2.5 (0–5)*	1 (0–3)
Med-Vat	0 (0–0)	5 (5– 8)*	8 (7–10)*	8 (6–10)	5 (4–7)*	5 (4– 7)*	3.5 (2–7)*	1.5 (0–3)
Med-Vat+ATI	0 (0–1)	5 (5–8)*	8 (7– 9)*	0.5 (0– 5)	0 (0–1)	0 (0–2)	0 (0–4)	0 (0–3)

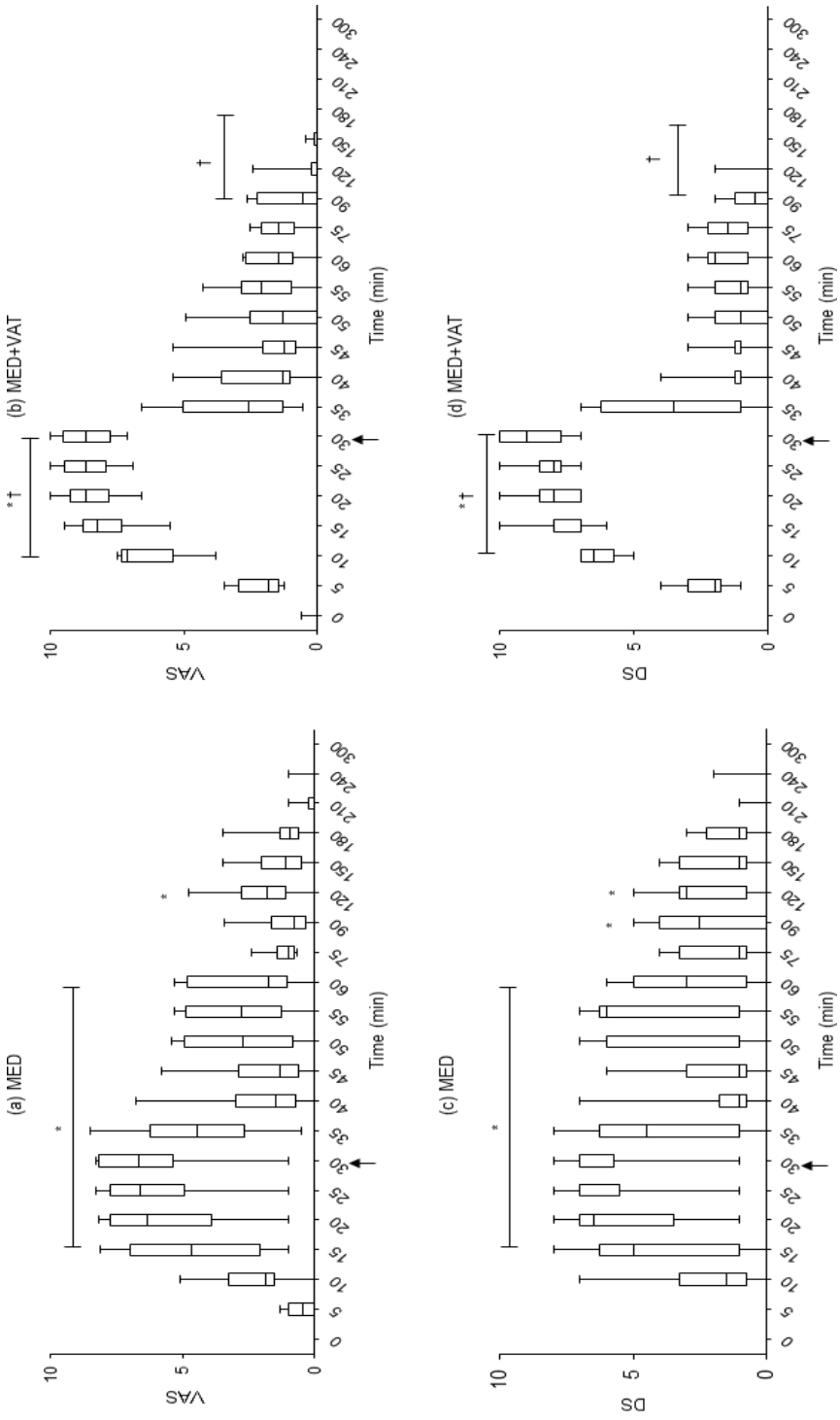

Figure 9. Median (line), interquartile range (box) and range (whiskers) scores for sedation using (a and b) a visual analog scale (VAS) and (c and d) a descriptive scale (DS) in eight sheep administered 30 mg/kg medetomidine (MED) and MED with 300 mg/kg of vatinoxan (MED+VAT) intramuscularly (IM), followed by atipamezole (150 mg/kg, IM) 30 minutes later (arrows). *Significantly different from baseline and †significantly different between treatments ($p < 0.05$).

6 DISCUSSION

6.1 Cardiopulmonary effects

In this series of studies, the effects of vatinoxan on cardiopulmonary function, plasma drug concentrations and clinical sedation were investigated in sheep treated with IM combinations of either medetomidine and ketamine or medetomidine alone (studies I–III). In general, following concomitant IM administration, vatinoxan dose-dependently alleviated most of the adverse cardiopulmonary effects associated with medetomidine and ketamine (study I). These results are consistent with the findings previously reported for sheep and dogs receiving vatinoxan combined with dexmedetomidine IV (Raekallio et al., 2010; Honkavaara et al., 2011), even though the study protocols were markedly different. However, in comparison with the IV studies, the early cardiopulmonary effects of medetomidine were not antagonized immediately (study I). Similar findings were reported for IM vatinoxan and medetomidine (Restitutti et al., 2017) and when the combination was further combined with butorphanol in dogs (Salla et al., 2014b). More specifically, in study I, HR and CO initially decreased significantly from baseline after all treatments, while MAP and CVP increased, except for the largest dose of vatinoxan (600 µg/kg). Likewise, PaO_2 decreased significantly for up to 30 minutes, nonetheless with vatinoxan it remained significantly higher than without it (study I). These findings could be attributed to the slower absorption rate of vatinoxan when compared with that of medetomidine after IM administration. In dogs, vatinoxan reached its T_{max} between 32–38 minutes, while the T_{max} for dexmedetomidine varied between 9–17 minutes after concurrent IM injection (Restitutti et al., 2017). Consequently, we can deduce that in studies I and II, the early concentration ratio between vatinoxan and dexmedetomidine in plasma was probably less than would have been necessary to reverse the medetomidine-induced cardiopulmonary effects during the initial period following treatment administration. For instance, in dogs anesthetized with isoflurane, a 50:1 ratio of vatinoxan:dexmedetomidine in plasma significantly antagonized the medetomidine-induced effects on HR and MAP, although with higher ratios hypotension occured (Kaartinen et al., 2014). However, in sheep, no hypotension was observed even with the highest dose (600 µg/kg) as MAP remained above 60 mmHg at all times (studies I and II). It is likely that anesthesia with isoflurane might have impaired the cardiovascular function resulting in an increased sensitivity to vatinoxan's vasodilatory action as reported by Kaartinen et al. (2014). In dogs and newborn lambs, isoflurane significantly decreased MAP in a dose-dependent manner (Brahim & Thut, 1984; Brett et al., 1987). In isoflurane-anesthetized dogs receiving vatinoxan (150 µg/kg IV) combined with medetomidine (10 µg/kg IV), MAP remained within clinically acceptable values (approximately 70 mmHg) (Salla et al., 2017), whereas with higher doses of vatinoxan (250 µg/kg IV) hypotension was observed (Salla et al., 2014a).

In study I, the addition of a small dose of ketamine to all treatments might have affected the pharmacodynamic interactions of vatinoxan and medetomidine. Ketamine is a cardiovascular stimulant, and when given IV (10 mg/kg) after xylazine (1 mg/kg, IV), it transiently, albeit significantly increased HR in xylazine-pretreated (1 mg/kg, IV) dogs. It also significantly increased CO, which nevertheless remained reduced when compared to values prior to xylazine administration (Haskins et al., 1986). Additionally, left ventricular work was increased, whereas SVR and left ventricle stroke index were significantly decreased after ketamine in xylazine-pretreated dogs, although SVR remained significantly above baseline values (Haskins et al., 1986). Furthermore, dogs

that received medetomidine (30 µg/kg) combined with ketamine (3 mg/kg) IM had a higher HR, compared with IM administration of medetomidine and butorphanol, even though all dogs were given glycopyrrolate as well (Ko et al., 2000). Similar findings were also reported for cats receiving ketamine and dexmedetomidine, 5 mg/kg and 10 µg/kg, IM, respectively, without any antimuscarinic treatment (Selmi et al., 2003).

In studies I and II, DO_2 decreased significantly from baseline after all treatments. Similarly, DO_2 was significantly reduced in dogs receiving medetomidine alone (Enouri et al., 2008; Rolfe et al., 2012) or when combined with butorphanol (Salla et al., 2014b) after both IV and IM injections. In isoflurane-anesthetized cats, no significant changes from baselines within treatments were detected in DO_2 after IM administration of dexmedetomidine (25 µg/kg) alone or when combined with vatinoxan (600 µg/kg), although a significant overall time effect was reported for both treatments (Siao et al., 2017). Oxygen delivery is a product of CO and arterial oxygen content (CaO_2), where the latter is calculated from the arterial Hb concentration and PaO_2. Consequently, any reduction in Hb content will negatively impact DO_2. Therefore, in the aforementioned cat study, despite the significant decrease in CI after dexmedetomidine alone, DO_2 remained statistically unchanged (Siao et al., 2017). The authors attributed this, among other probable reasons, to the increase in arterial Hb concentration after dexmedetomidine, which appeared to counteract the effect of the reduction in CI on DO_2 (Siao et al., 2017). In studies I and II, the decreases in CO were small both in the presence and absence of vatinoxan. Therefore, the induced changes in DO_2 can be attributed mainly to the reductions in Hb concentrations (and consequently, on CaO_2) even though CO tended to be higher with vatinoxan than without it (studies I and II). In medetomidine-treated dogs (Enouri et al., 2008; Rolfe et al., 2012) both CI and DO_2 decreased significantly either with or without vatinoxan, although CI was lower without the antagonist. However, with vatinoxan the oxygen extraction ratio (ER) remained within baseline values. The authors postulated, that due to the higher CI, addition of vatinoxan still maintained a better supply of oxygen to tissues as the increase in ER indicates a proportionate increase in demand versus supply (Rolfe et al., 2012). In studies I and II, however, the ER was not calculated due to lack of mixed-venous partial oxygen tension measurements. However, with all treatments, plasma lactate concentrations did not increase which would imply that tissues were adequately perfused and there was no tissue hypoxia. Lactate production, and thus its concentration in blood, increases as a result of a decreased cellular oxygen delivery. This relationship between increased lactate levels in blood and presence of an oxygen debt was first described by Meakins & Long (1927). Clinically, lactate levels in blood are frequently used to monitor tissue hypoxia.

In study IV, when vatinoxan was given IV 10 minutes before dexmedetomidine, it prevented all the adverse pulmonary changes in sevoflurane-anesthetized sheep. As such, pulmonary edema can be defined as an accumulation of extravascular fluid in the lungs, most typically within the alveoli. Traditionally, two types of pulmonary edema are recognized, depending on the underlying cause: hemodynamic (cardiogenic) edema due to increased hydrostatic pressure, and permeability (non-cardiogenic) edema due to increased endothelial permeability (Staub, 1974; Murray, 2011). Hydrostatic pulmonary edema associated with increases in MPAP, PAOP and pulmonary capillary pressure (P_C) has been observed in sheep receiving dexmedetomidine (Kästner et al., 2007). On the other hand, the efflux of proteinaceous fluid into the alveolar spaces following ST-91, an α_2-adrenoceptor agonist that does not cross the blood-brain barrier, suggested altered permeability (Celly

et al., 1999b). In study IV, CT imaging suggested that the lung densities were increased following the administration of dexmedetomidine alone. This effect is comparable to the findings reported previously for sheep receiving 2 µg/kg IV dexmedetomidine (Kästner et al., 2007). However, the changes observed in study IV were less pronounced and developed more slowly than those reported by Kästner et al. (2007). Moreover, no abnormalities such as foamy fluid were evident during the bronchoscopy (study IV). Conversely, the post-mortem examination of sheep lungs with pulmonary edema revealed that the tracheas were filled with foamy fluid (Kästner et al., 2007). This inconsistency between the previous results and ours might be due to variation in individual sensitivity to α_2-adrenoceptor agonists, even between individuals within the same breed. In addition, the use of PEEP during mechanical ventilation in study IV might have altered dexmedetomidine's effects. In experimental canine and porcine models of induced pulmonary edema, the use of PEEP (10–20 cmH_2O) limited the increase in pulmonary extravascular fluid (Fernández-Mondéjar et al., 1996; Ruiz-Bailén et al., 1999), and significantly improved gas exchange and decreased the shunt fraction when compared to zero end-expiratory pressure (Wickerts et al., 1992). The application of PEEP effectively decreases the shunt fraction by recruitment of collapsed or unventilated sections of the lungs (Wickerts et al., 1992), increases total and/or regional functional residual capacity, and together with the subsequent prevention of the intermittent airway closure and opening, reduces the alveolar-to-arterial pO_2 gradient and venous admixture (McDonell & Kerr, 2015). Therefore, in study IV, although only 5 cmH_2O of PEEP was used, it might have prevented detectable vascular changes and limited the formation of pulmonary edema. Further, since most of the CT changes were seen in the dependent, ventral part of the lungs, this could partially explain the lack of changes in BAL analyses as they were performed from the dorsal and accessory lung lobes. Additionally, the changes in CT could have been mainly due to the formation of interstitial rather than alveolar edema (study IV). It is also worth mentioning that the changes in PaO_2 observed in studies I, II, and IV seemed to be reversible. In previous studies in sheep, later IV administration of α_2-adrenoceptor antagonists (15–20 minutes after the agonist), failed to completely reverse the hypoxemia induced by xylazine and medetomidine (Doherty et al., 1986; Ko & McGrath, 1995). Again, the variation in the extent of the induced morphological pulmonary changes might explain this discrepancy between the studies. Evident alveolar edema and hemorrhage were observed in sheep lungs 60 minutes after receiving xylazine (150 µg/kg, IV), and even 12 hours later a few areas of alveolar edema and hemorrhage persisted, suggesting a more pronounced pulmonary pathology when compared to the present studies I, II and IV (Celly et al., 1999a). In general, vatinoxan appeared to attenuate the cardiopulmonary changes induced by medetomidine after IM co-administration and also prevent dexmedetomidine-induced pulmonary changes when administered as an IV pre-treatment. However, in conscious sheep, the improvement in cardiopulmonary function was somewhat lessened by a decrease in hemoglobin concentrations negatively impacting oxygen delivery. Moreover, presence of vatinoxan hastened complete reversals of medetomidine-induced cardiopulmonary changes by atipamezole as the two antagonists seemed to interacted favorably, without adverse effects in sheep sedated with medetomidine and/or medetomidine and ketamine.

6.2 Plasma concentrations of drugs

After concurrent IM administration in the same syringe, vatinoxan increased the early plasma concentrations of dex- and levomedetomidine (studies II and III). This finding is consistent with

reports in dogs (Restitutti et al., 2017) and cats (Pypendop et al., 2017b), as vatinoxan roughly doubled the C_{max} of dexmedetomidine and reduced its T_{max} in dogs (Restitutti et al., 2017) and cats (Pypendop et al., 2017b). This accelerated absorption of medetomidine probably resulted from reversal of the medetomidine-induced local vasoconstriction at the site of injection, as suggested by Restitutti et al. (2017). Moreover, vatinoxan approximately halved the T_{max} for other sedative agents, butorphanol and midazolam, after single-syringe IM co-administration in dogs (Kallio-Kujala et al., 2018a). Interestingly, the aforementioned effect of vatinoxan on medetomidine concentrations was not detected after co-administration with ketamine (study I). This could possibly be attributed to a direct vasodilatory effects on the peripheral vasculature by ketamine, mimicking the local effects of vatinoxan (White et al., 1982). Further, the acidity of the ketamine solution (pH 3.5–5.5) might have changed the mixture's pH. Medetomidine pKa is 7.1 which means that at pH 7.1 its base and salt forms will be present in a 50:50 ratio. Thus, the percentage of non-ionized medetomidine could be increased at acidic pH and subsequently increases its absorption rate. Also, addition of the ketamine preparation may have changed the solution's physicochemical properties that could have led to the increase of medetomidine absorption. No reports are available concerning effects of ketamine on the absorption of α_2-adrenoceptor agonists or other drugs after IM administration. In cattle, sheep (Deghani et al., 1991), and blue foxes (Jalanka, 1990) the onset of sedation following IM administration of xylazine or medetomidine combined with ketamine was faster when compared with the α_2-adrenoceptor agonist alone, although this may have been due to additional central depressive effects by the dissociative. In studies II and III, blood samples for drug concentration analyses were not collected frequently enough to precisely determine the absorption phase so comparing absorption kinetics between studies I, II and III is not possible. However, the plasma concentrations of dex-, levomedetomidine, and vatinoxan seemed higher in study I than when measured in studies II and III. For instance, with Med-Ket treatment in study I the plasma concentrations of dexmedetomidine at T20 and T30 were significantly higher than those in studies II and III after medetomidine alone. A comparable effect of ketamine on vatinoxan was observed as the plasma concentrations of vatinoxan with the middle dose in study I (Med-Ket-Vat300) at T20 and T30 seemed to be higher than with Med-Vat and MED+VAT in studies II and III respectively. However, the difference was not statistically significant. Additionally, in study II, the semimembranosus muscle was used for treatment administration, whereas the shoulder muscles were the assigned muscle group in studies I and III. The activity and blood flow of the muscle affect drug bioavailability (Buxton, 2006). The postural muscles usually have more abundant blood flow that hastens drug absorption compared with nonpostural muscles (Baxter & Evans, 1973). Moreover, the amount of peri-muscular fat or intermuscular fascial planes can decrease the rate of drug absorption (Sund & Schou, 1964). In dogs, for instance, the onset of sedation was faster, and its quality was higher after IM dexmedetomidine and hydromorphone administered into the semimembranosus and cervical muscles compared with lumbar and gluteal ones (Carter et al., 2013). Furthermore, in dairy cows, amoxicillin absorption from *M. triceps* was better than from the buttocks (Rutgers et al., 1980). No such data comparing the effect of IM injection sites on the absorption of sedative drugs in sheep are available.

On the other hand, vatinoxan significantly increased the CL of (dex-)medetomidine following simultaneous IV administration in dogs (Honkavaara et al., 2012; Bennett et al., 2016). Similarly, in cats, dexmedetomidine CL was significantly higher with vatinoxan after both IV (Pypendop et al., 2016) and IM (Pypendop et al., 2017b) administration when compared with the agonist alone. In

study II, though our follow-up period was relatively short compared with some of the previously mentioned studies, vatinoxan seemed to enhance the CL of medetomidine. The plasma concentrations of dex- and levomedetomidine were significantly lower in the presence of vatinoxan from 60 and 90 minutes onwards in studies II and III, respectively. Arguably, increased biotransformation related to enhancement of hepatic blood flow by vatinoxan could partly explain these differences although it was not directly evaluated during our studies.

In studies I–III, plasma concentrations of dex- and levomedetomidine appeared nearly identical, regardless of the presence or absence of vatinoxan, ketamine or atipamezole. Conversely, in dogs, dexmedetomidine concentrations were significantly higher than those of levomedetomidine after both IV (Bennett et al., 2016, 2017) and IM (Kallio-Kujala et al., 2018a) administration of the racemic mixture, which should theoretically contain equal amounts of both enantiomers. However, when the pharmacokinetics of medetomidine (40 µg/kg) and its enantiomers, dexmedetomidine (20 and 10 µg/kg) and levomedetomidine (20 and 10 µg/kg), were compared after IV administration in dogs (Kuusela et al., 2000), no significant differences in CL, V_{SS}, or $T_{½}$ were detected between medetomidine and dexmedetomidine. That said, the CL and V_{SS} of levomedetomidine were significantly higher and larger than with dexmedetomidine and the racemic medetomidine, while the $T_{½}$ did not significantly differ between the enantiomers (Kuusela et al., 2000). Nevertheless, in the aforementioned study, all the drugs were administered separately, which means that levomedetomidine's pharmacokinetics were determined without the cardiovascular depressant effect of dexmedetomidine (Kuusela et al., 2000). However, in dogs treated with racemic medetomidine (10 µg/kg IV), the $T_{½}$ of dexmedetomidine was significantly lower than that of levomedetomidine, whereas the AUC and concentrations of dexmedetomidine in plasma were significantly higher than for the levoisomer (Bennett et al., 2016). Further, in dogs, after administration of medetomidine, the clearance of levomedetomidine was significantly higher than that of dexmedetomidine, both in the presence and absence of vatinoxan (Bennett et al., 2016; Kaartinen, 2016). Consequently, as no such differences were noted, we can deduce that in sheep the clearance of the enantiomers seems to differ less than in dogs.

Administration of atipamezole resulted in marked increase in both dex- and levomedetomidine concentrations in plasma (studies II and III). For instance, with medetomidine alone, concentrations of dex- and levomedetomidine were approximately tripled 10 minutes after atipamezole (study II). However, in the presence of vatinoxan, pre-reversal concentrations were not exceeded (study II). This difference could be attributed to the earlier achievement of C_{max} in the presence vatinoxan, as mentioned. Nevertheless, the increase in medetomidine concentration after atipamezole administration is a well-documented phenomenon in ruminants (Ranheim et al., 1997; 1998; 1999; 2000b). For example, in sheep receiving IV atipamezole (200 µg/kg) 60 minutes after IV medetomidine (40 µg/kg), the maximum increase (approximately two- to three-fold) in medetomidine concentrations in plasma was detected 5 minutes after atipamezole administration and they remained elevated between 25 to 45 minutes before regaining pre-reversal concentrations (Ranheim et al., 2000b). This phenomenon has not been observed in other species, such as dogs (Salonen et al., 1995), horses (Knych et al., 2012), ponies (Dyer et al., 1987) or humans (Scheinin et al., 1998). Presumably, atipamezole might have displaced medetomidine from highly perfused organs such as kidneys, CNS, lungs and liver thus increasing its concentration in plasma, as previously suggested by Ranheim et al. (1998; 1999; 2000b). Furthermore, atipamezole might have increased the absorption of medetomidine

from its injection site, due to improvement of global cardiovascular function (study II). In contrast, after medetomidine alone its concentrations in plasma were stable throughout the follow-up period, suggesting equal absorption and elimination rates, or a flip-flop phenomenon (study II). Further, in sheep receiving IM medetomidine (30 μg/kg), the T_{max} ranged between 15-40 minutes and C_{max} was 4.98 ± 1.9 ng/mL (Kästner et al., 2003), which possibly reflected a slower absorption rate from the assigned muscle group in study II compared to ones used by Kästner et al. (2003). On the other hand, in study I, the T_{max} was shorter than reported earlier (32.5–38.1 minutes) for dogs receiving various doses of IM vatinoxan in combination with medetomidine (Restitutti et al., 2017). Administration of atipamezole had no significant effect on vatinoxan concentrations in plasma (studies I–III), nor were the concentrations of atipamezole affected by the presence of vatinoxan (studies I–III). On the contrary, in dogs, vatinoxan significantly decreased plasma concentration of atipamezole and reduced its AUC in dogs receiving medetomidine (Turunen et al., 2017).

6.3 Clinical sedation

The AUCs of sedation scores from study I showed no significant differences in the level of sedation between treatments regardless of the addition of vatinoxan, which was probably attributed to ketamine as discussed earlier. However, the lowest dose of vatinoxan (150 μg/kg) did not completely reverse the adverse cardiopulmonary effects of Med-Ket. The highest dose (600 μg/kg), on the other hand, provided no additional benefits and two animals passed abnormally soft feces. Thus, the middle dose of vatinoxan (300 μg/kg) appeared to be the optimal one for concurrent IM administration with 300 μg/kg medetomidine. Addition of that dose of vatinoxan to medetomidine produced a more rapid onset of sedation in sheep than with medetomidine alone (studies II and III). This finding is consistent with previous studies in dogs (Restitutti et al., 2017; Kallio-Kujala et al., 2018a, b) and cats (Honkavaara et al., 2017b). In dogs, the time to peak sedation was in line with the T_{max} of dexmedetomidine (Kallio-Kujala et al., 2018a). These increased sedation scores could be due to the accelerated absorption of medetomidine from the injection site by the addition of vatinoxan, resulting in an earlier and higher C_{max} for medetomidine. Restitutti et al. (2017) suggested that vatinoxan might have reversed the local vasoconstriction induced by medetomidine. On the other hand, from 90 minutes onwards, the level of sedation was higher after medetomidine alone than when vatinoxan was added (study II). These findings seemed to directly reflect the plasma concentrations of dexmedetomidine. Dexmedetomidine is also known to have a ceiling effect above which increasing the dose (or actually concentration in plasma) does not further deepen the sedation. For example, in domestic cats receiving a stepwise infusion of medetomidine, the depth of sedation was dose-dependent up to certain serum concentration (45 ng/mL, dexmedetomidine) and beyond that level the sedation degree was decreased (Ansah et al., 2000). Further, in sheep, the observed degree of sedation correlated well with the concentration in plasma after IM medetomidine (30 μg/kg) at the peak plasma concentration and a T_{max} of (range) 15–40 minutes (Kästner et al., 2003). Similarly, after IV medetomidine (15 μg/kg and 40 μg/kg) the sedative effects declined in parallel with plasma medetomidine concentrations (Muge et al., 1996; Ranheim et al., 2000b). In contrast, in some other ruminants such as cattle and reindeer, medetomidine concentrations appeared to correlate less directly with its sedative effects (Ranheim et al., 1997, 1998, 1999). Overall, it seemed that the degree of induced sedation directly represented (dex-)medetomidine concentrations in plasma. In study I, for

example, one animal showed a low sedation scores after Med-Ket treatment, which was associated with low dexmedetomidine plasma concentrations.

Reversals with atipamezole were smooth and uneventful both with or without vatinoxan (studies II and III). In contrast, in study I, most of the animals showed signs of agitation, excitement and facial muscle tremors after atipamezole administration. The timing of atipamezole administration (30 minutes after medetomidine in studies II and III versus 60 minutes after medetomidine-ketamine in study I) might explain these differences, because the longer interval unsurprisingly lead to lower plasma dexmedetomidine concentrations at the time of reversal, favoring the clinical effects of atipamezole. In a previous study in sheep (Ranheim et al., 2000b), all the animals exhibited similar over-alertness and muscle tremors when atipamezole (200 µg/kg IV) was administered 60 minutes after medetomidine (40 µg/kg IV). On the other hand, sheep appeared alert when sedation was reversed by atipamezole (200 µg/kg IV) immediately after the animals had become recumbent 9–25 minutes after 40 µg/kg IM medetomidine was given (Mohammad et al., 1995). Thus, we can conclude that when the sedative effects of medetomidine have already been considerably dissipated, a lower dose of atipamezole might be preferable. This conclusion is supported by earlier results of Talke et al. (2000), who reported that administration of atipamezole at a dose 2.5 times that of a preceding medetomidine dose in sheep, no excitement or agitation was observed. Moreover, when IV atipamezole (30 or 60 µg/kg) was given to lambs 15 minutes after medetomidine (30 µg/kg IM), all the animals recovered and walked unassisted in 2.4 ± 0.4 and 2.3 ± 0.7 minutes respectively (Ko & McGrath, 1995). Additionally, the possible residual effects of ketamine at the time of atipamezole administration may have contributed to the observed agitations and tremors in study I.

Following the initial recovery after atipamezole, some of the animals treated with medetomidine alone (studies II and III) or medetomidine-ketamine (study I) became lethargic and seemed drowsy. With the addition of vatinoxan, fewer animals seemed to exhibit these signs. Honkavaara et al. (2008) postulated that the presence of vatinoxan might have enhanced the central bioavailability of atipamezole by decreasing the amount of atipamezole bound to peripheral α_2-adrenoceptors, thus leading to more complete reversals. However, in an earlier study, no relapse into sedation was observed after the reversal of medetomidine-induced sedation by atipamezole in sheep (Ranheim et al., 2000b). In contrast, in dairy calves and cattle, resedation was evident approximately 80 minutes after atipamezole administration (Ranheim et al., 1998; 1999), and between 30–60 minutes after atipamezole injection to reindeer (Ranheim et al., 1997). In free-ranging cattle sedated with medetomidine, resedation was detected between 1–2 hours and 3–4 hours after IV and IM atipamezole, respectively (Arnemo & Soli, 1993b; 1995). In studies I and II, the assessment of resedation was compromised by the use of a sling, the concurrent physiologic measurements, and the relatively short observational period after reversal (60 minutes in study I and 90 minutes in study II). Therefore, in study III, all sedation assessments were performed from a distance, without approaching or interacting with the animals. However, when the animals became sternally recumbent, it became challenging to recognize resedation. Overall, vatinoxan appeared to ensure more complete recoveries when atipamezole was used to reverse medetomidine-elicited sedation. The rapid onset of sedation and lack of resedation after reversal are advantageous, especially with free-ranging and non-domesticated animals.

6.4 Other effects

6.4.1 Rectal temperature

In study I, rectal temperature decreased significantly after all treatments. Even after reversal with atipamezole, it remained below the baseline, although remaining within an acceptable physiological range (38.3–39.9°C). Similar results were reported in calves after sedation with medetomidine and ketamine (Raekallio et al., 1991) and medetomidine alone followed by atipamezole (Rioja et al., 2008). Decreases in temperature after the use of α_2-adrenoceptor agonists are a well-documented phenomenon (Ponder & Clark, 1980; Virtanen, 1989; Keegan et al., 1995; Pypendop et al., 1996; Honkavaara et al., 2017b). On the other hand, a rise (Ranheim et al., 1999; Rioja et al., 2008) or no change (Mohammad et al., 1993; 1995) in core temperature have also been reported. The reduction in temperature after α_2-adrenoceptor agonists is attributed to CNS depression and decreases in muscular activity (Sinclair, 2003). Moreover, a proposed biphasic effect of medetomidine on rectal temperature has been suggested, characterized by a brief, initial rise as a result of peripheral vasoconstriction followed by a prolonged decrease due to the reduced muscular activity (Kuo & Keegan, 2004). This proposed biphasic effect is supported by the finding in dogs of Rolfe et al. (2012), where the concurrent administration of vatinoxan with medetomidine prevented the initial increase in rectal temperature but not the subsequent decrease, presumably by preventing the peripheral vasoconstriction. In dogs sedated with medetomidine and butorphanol, the addition of vatinoxan resulted in a significant increase in superficial temperatures measured by thermography, accompanied by a significant decrease in rectal temperature (Vainiopää et al., 2013a). This suggested that the enhancement of peripheral blood flow by vatinoxan led to an increase in peripheral heat loss (Vainiopää et al., 2013a). Jalanka & Roeken (1990) suggested that hypo- or hyperthermia could develop if immobilized animals are exposed to extreme ambient temperature for long periods. For instance, at room temperature, rectal temperatures remained stable in snow leopards (Jalanka, 1989) and blue foxes (Jalanka, 1990) receiving medetomidine, but in markhors immobilized with medetomidine-ketamine at ambient temperatures between 5 and 12°C, they were significantly decreased (Jalanka, 1988). In cats, xylazine-induced hypothermia developed more rapidly when animals were placed in a cold environment (4°C), whereas at hot ambient temperature (32°C) a mild hyperthermia developed instead (Ponder & Clark, 1980). Therefore, it could be concluded that α_2-adrenoceptor agonists seem to affect thermoregulation rather than the body temperature itself. Consequently, the aforementioned variation more likely depends on the ambient temperature. In our studies, no increase in rectal temperatures were recorded regardless of the presence or absence of vatinoxan. However, we might have missed the initial changes in body temperature because the first measurements were made 15 and 10 minutes after the treatments were administered in studies I and II, respectively. Further, the studies were carried out at an ambient room temperature of 20–22°C, with no other further efforts aimed at maintaining normothermia. However, vatinoxan may predispose animals to more pronounced changes in core temperature as they may become more vulnerable to the surrounding conditions.

6.4.2 Hemoglobin content

Blood hemoglobin concentrations were significantly decreased from baseline with all treatments (studies I and II). However, with the treatments without vatinoxan, Hb concentration remained higher (studies I and II). Likewise, Salla et al. (2014a) reported that the Hb concentration was significantly

lower in dogs treated with medetomidine and vatinoxan than that after medetomidine alone. Thus, vatinoxan might have antagonized the peripheral effect of medetomidine on the splenic capsule, as (dex-)medetomidine alone has been reported to increase Hb concentration in dogs (Rolfe et al., 2012) and cats (Siao et al., 2017). In horses, on the other hand, α_2-adrenoceptor agonists caused a marked decrease in packed cell volume (Gasthuys et al., 1990; Wagner et al., 1991; Kullmann et al., 2014) and Hb concentrations (El-Kammar & Gad, 2014), associated with increases in splenic thickness (Kullmann et al., 2014). Any decrease in sympathetic tone can lead to splenic relaxation and consequently red blood cell sequestration in horses (Wagner et al., 1991; Kullmann et al., 2014). The lack of change in study IV after either treatment could be attributed to the relatively small agonist dose used. By itself, sevoflurane unlikely affected the Hb concentration because no changes were detected in Hb levels in humans that inhaled 1 MAC sevoflurane (Bozdogan et al., 2005). In any case, the more pronounced decrease in Hb concentrations associated with vatinoxan decreased CaO_2, as discussed earlier. Reversal with atipamezole returned Hb concentrations to baseline levels with all treatments (studies I and II).

6.4.3 Plasma glucose concentration

The hyperglycemic effect of medetomidine observed in studies I, II, and IV was expected as it is a well-documented phenomenon in several species following the use of various α_2-adrenoceptor agonists (Hsu & Hummel et al., 1981; Burton et al., 1997; Ranheim et al., 2000a; Carroll et al., 2005). Vatinoxan prevented the increase in plasma glucose and maintained its concentrations close to baseline levels (studies I, II, and IV). Similar findings were reported for dogs receiving IV dexmedetomidine combined with vatinoxan, where the antagonist prevented the dexmedetomidine-induced decrease in plasma insulin concentrations and the consequent hyperglycemia (Restitutti et al., 2012). Also, in horses, vatinoxan partially prevented romifidine-evoked hyperglycemia (Pakkanen et al., 2018). In studies I and II, administration of atipamezole did not reverse the effects of medetomidine on plasma glucose. This is consistent with an earlier finding in sheep where atipamezole did not reverse the primary hyperglycemia but prevented the secondary further increase in glucose concentration (Ranheim et al., 2000a). In contrast, in dairy cows and calves, atipamezole clearly antagonized medetomidine-induced hyperglycemia (Ranheim et al., 2000a).

6.5 Methodological considerations and study limitations

We used only intact female sheep in this series of investigations. However, sex predilection is an unlikely cause of differences in the hypoxemic response to α_2-adrenoceptor agonists in this species (Nolan et al., 1990; Celly et al., 1997a). However, the individual variability in response to α_2-adrenoceptor agonists is common among sheep. Breed differences was suggested as a cause of dissimilarity in sheep analgesic response to IV xylazine (Ley et al., 1990). On the other hand, extensive individual variation in the hypoxemic response to medetomidine has been reported (Tulamo et al., 1995). Moreover, Kästner et al. (2007) reported that out of seven sheep that received IV dexmedetomidine, one showed only minimal systemic and pulmonary vasoconstriction during the study, which was later confirmed by the lack of pulmonary capillary disruption in the post mortem examination. In contrast, the same group (Kästner et al., 2003) reported that one individual sheep out of nine became hypoxemic with PaO_2 decreased below 60 mmHg after IM medetomidine (30 µg/kg), although there were no differences in medetomidine pharmacokinetics detected between animals. In

accordance, we noticed substantial individual variability in PaO_2, although severe hypoxemia was only sporadically noted. Among the animals used in the present studies, there were some individuals whom exhibited low PaO_2 across the treatments while others did not. In study IV, for instance, in two sheep, PaO_2 was more than halved from baseline after dexmedetomidine alone, although administration of vatinoxan before dexmedetomidine prevented the decrease in PaO_2 in all individuals.

Two distinct Hb types (Hb A and Hb B) are commonly found in adult sheep (Evans et al., 1956; 1958). Accordingly, three types of animal can be distinguished based on their Hb type, i.e. A, B, or AB. The PaO_2 at which Hb is half saturated (P_{50}) varies among sheep depending on their Hb type. Maginniss et al. (1986) reported that P_{50} was 31 mmHg for sheep with Hb A, while it was 40 and 35 mmHg for Hb B and Hb AB sheep respectively. However, in the present study no Hb characterization was performed. Thus, in studies I and II, the standard formula for Hb AB was used for calculation of saturation indices, whereas in study IV, no saturation calculations were performed and SpO_2 was recorded with pulse oximetry. Moreover, the accuracy of Hb concentrations in studies II and IV might be questioned because they were obtained from blood gas analyses performed by analyzers using algorithms designed for humans. However, the studies aimed to compare effects between treatments and all samples were analyzed similarly. Additionally, a similar treatment effect was observed on Hb in study I when the samples were analyzed using a hematology analyzer validated for sheep. Furthermore, the repeated blood sampling for LiDCO, plasma drug concentrations, and blood gas analyses, might have affected the Hb concentration, blood volume and subsequently the cardiopulmonary parameters. However, the maximum blood loss during the studies was much less than 10% of the estimated total blood volume, with 14 days of rest between each two consecutive trials (Morton et al., 1993; Diehl et al., 2001). Therefore, the blood loss and IV fluid replacement were unlikely to markedly affect the results as compared to the effect of treatments.

In studies I, II and IV, CO was measured using the lithium dilution method. In anesthetized sheep, lithium dilution was suggested to be biased when tested against a pulmonary arterial flow detector probe as it did not detect changes in CO reliably enough. However, the results were comparable to those reported for other animal studies (Axiak Flammer et al., 2013). Nevertheless, in anesthetized pigs, lithium dilution performed better than thermodilution when the accuracy of both methods were compared against a flow detector probe (Kurita et al., 1997). In dogs, LiDCO and thermodilution measurements correlated well (Mason et al., 2001). Also, in dogs with experimentally induced, normo-, hypo-, and hyperdynamic hemodynamic conditions, there was a good agreement between LiDCO and thermodilution (Morgaz et al., 2014). *In vitro*, medetomidine and ketamine can react with the lithium sensor and increase its voltage, which can affect the reported values (Ambrisko et al., 2013). However, the magnitude of this effect is concentration dependent. In our studies, concentrations of medetomidine and ketamine in plasma were much lower than those that reportedly cause any appreciable bias: only the ketamine concentration in study I was > 300 ng/mL, which can induce a bias of 1.9% (Ambrisko et al., 2013). Moreover, *in vivo* biases with clinically relevant plasma concentrations have been reported for some drugs, such as xylazine in horses (Ambrisko & Moens, 2014), but atipamezole does not markedly interfere with the performance of the lithium sensor (Ambrisko et al., 2013). However, no data are available on the interaction between vatinoxan and the lithium sensor, thus we are not able to determine whether this may have influenced the accuracy of CO measurements. Overall, as the aforementioned studies were performed either *in vitro* or in species

other than sheep, therefore it is difficult to estimate the potential influence of the drugs used on the lithium sensors. It seems unlikely that these interactions would have had a significant impact compared with the effect of vatinoxan on CO, which is well documented in other species and using other methods (Enouri et al., 2008; Rolfe et al., 2012; Siao et al., 2017; Martin-Flores et al., 2018). However, a further investigation would be warranted to determine the potential effects of vatinoxan on lithium sensor voltage which may bias the CO measurements. Additionally, any cumulative increase in blood lithium concentration might affect the accuracy of CO measurements. The manufacturer determined a serum lithium concentration of 0.2 mmol/L as a cutoff value, above which an overestimation of CO due to background lithium is expected. However, in the present study, residual background lithium was an unlikely cause for any bias since no more than seven lithium injections of 0.075 mmoL each were given during a trial. In dogs, for example, 34 lithium doses within the span of 3–7 hours are needed to reach 0.2 mmol/L limit (Mason et al., 2002).

In studies I and II, the sheep were placed in a sling that kept them in an upright position with limited ability to move, which may have impaired the assessment of sedation and its reversal. Additionally, the heavy instrumentation and the simultaneous cardiopulmonary measurements might have affected the overall degree of sedation. Therefore, study III focused primarily on sedation evaluation with the animals unrestrained and allowed to behave normally without interference.

In studies I, II, and IV, the blood samples for plasma drug concentrations were collected from the carotid artery, whereas in study III (due to lack of arterial cannulation) the samples were obtained from the jugular vein. The drug concentrations in venous blood samples may vary depending on the vein being sampled (Chiou, 1989) and sometimes they are proportional to the drug concentration in the tissues drained by that vein (Lam et al., 1982). Arterial blood samples, on the other hand, are independent of sampling site and generally preferred when studying the relationship between plasma concentrations and effect (Chiou, 1989). In cats, for instance, jugular venous blood sampling resulted in an overestimation of plasma buprenorphine concentrations after buccal administration compared to carotid arterial blood sampling; however, no difference between the two sites was observed in calculated pharmacokinetic parameters following IV administration (Hedges et al., 2014). In the present study, it was unlikely that the sampling site markedly affected the plasma drug concentrations since the plasma concentrations in studies II and III were comparable. Further, the samples in study III were collected from the upper third of the jugular vein, while the shoulder muscles venous drainage ends up in the axillary vein which drains into the subclavian vein to cranial *Vena cava* (Schummer et al., 1981).

6.6 Clinical relevance and future prospects

When administered intramuscularly in the same syringe, vatinoxan dose-dependently alleviated the cardiopulmonary effects of medetomidine without compromising sedation. In fact, vatinoxan appeared to shorten the onset of sedation and was also associated with more complete recoveries after reversal with atipamezole. These findings might prove very useful, especially in field conditions. Apart from sheep, vatinoxan could enhance the safety of medetomidine and medetomidine-ketamine sedation in various non-domestic ruminant species, although re-evaluation of the optimal doses would be necessary. In sheep, as the current studies were experimental and performed under well-controlled laboratory settings, larger clinical field trials would consequently remain warranted. Moreover,

further elucidation of the pharmacokinetic-pharmacodynamic interactions between vatinoxan, medetomidine, atipamezole and other sedatives, anesthetics and analgesics are required to describe clinically relevant outcomes such as anesthetic-sparing, antinociception and gastrointestinal function both in sheep and other ruminant species.

The present studies revealed that vatinoxan dose-dependently counteracted the hyperglycemic effect of (dex-)medetomidine. Nevertheless, the study design investigated only clinically healthy individuals and metabolic outcomes were not prioritized. Therefore, a further elucidation of vatinoxan's effects on metabolic changes prompted by α_2-adrenoceptor agonists in sheep requires future research, especially in compromised or stressed animals and those with underlying diseases. Additionally, study designs including a vatinoxan only treatment arm would have added more detailed information regarding its metabolic effects in sheep.

7 CONCLUSIONS

1. Vatinoxan dose-dependently attenuated most of the cardiopulmonary suppression in conscious sheep sedated with medetomidine and ketamine. The presence of vatinoxan hastened the reversal by atipamezole.

2. Vatinoxan increased the early concentrations of medetomidine in plasma when co-administered IM in the same syringe.

3. Vatinoxan had no significant effect on ketamine concentrations, when both were administered in combination with medetomidine, or on atipamezole concentrations administered later for sedation reversal.

4. Vatinoxan increased the intensity of clinical sedation induced by medetomidine following concurrent IM administration, whereas it had no clinically relevant effect on the sedation scores produced by a medetomidine and ketamine combination. Vatinoxan ensured more complete recoveries when atipamezole was used to reverse medetomidine or medetomidine and ketamine-induced sedation.

5. Pre-treatment with vatinoxan prevented dexmedetomidine-induced pulmonary alterations such as pulmonary edema formation and increase in airway resistance while reducing the decreases in PaO_2 in sevoflurane-anesthetized sheep.

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