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Impact of vatinoxan on cardiopulmonary and gastrointestinal effects of medetomidine and detomidine in horses

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ACADEMIC DISSERTATION

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To my family

ABSTRACT

Detomidine and medetomidine are α_2-adrenoceptor agonists that are used in equine medicine for sedation and analgesia. These drugs, however, also influence the cardiopulmonary and gastrointestinal system, mainly by increasing systemic vascular resistance and decreasing cardiac performance and intestinal motility. Vatinoxan, in turn, is primarily a peripherally acting α_2-adrenoceptor antagonist that has been demonstrated to attenuate some of the undesired effects of α_2-adrenoceptor agonists with no major influence on the level of sedation in many species.

One aim of the present studies was to evaluate the effects of vatinoxan with a constant rate infusion (CRI) of medetomidine in standing and anesthetized healthy horses. Another aim was to evaluate the effects of vatinoxan when it is administered after detomidine in standing healthy horses. We hypothesized that vatinoxan would alleviate the cardiopulmonary and gastrointestinal effects of these α_2-adrenoceptor agonists, while preserving their sedative effects under these experimental conditions.

The horses received, in a cross-over design in three separate studies, an α_2-adrenoceptor agonist either alone or with vatinoxan for sedation or for premedication before general anesthesia. To evaluate the cardiopulmonary function, the heart rate, respiratory rate, cardiac output, arterial blood pressures, central venous pressure, and pulmonary arterial pressures (this last one in two of the studies) were recorded. Additionally, arterial and venous blood gases were analyzed. Systemic vascular resistance, oxygen delivery, and other selected cardiopulmonary parameters were calculated afterwards. The effects of the drugs on intestinal motility were evaluated by auscultation of the borborygmi or by determining the amount of fecal output. The level of sedation was scored, and the recovery times were recorded in anesthetized horses. Finally, the drug concentrations in plasma were determined.

In standing horses receiving a CRI of medetomidine, vatinoxan attenuated the early, although minor, cardiopulmonary changes induced by medetomidine. Furthermore, the intestinal motility was markedly improved by vatinoxan. In anesthetized horses receiving an adjunctive CRI of medetomidine, premedication with vatinoxan induced significant hypotension. Despite this hypotension, which was successfully treated with dobutamine, the cardiac performance and tissue oxygen delivery were better maintained in these horses than in those receiving the adjunctive medetomidine CRI without vatinoxan. The fecal output decreased after general anesthesia, and this decrease was not influenced by vatinoxan. Finally, vatinoxan, administered after detomidine, partially reversed the cardiopulmonary effects and intestinal hypomotility induced by detomidine. Vatinoxan decreased the level of sedation at the beginning of the CRI of medetomidine in standing horses, but not when administered with a single dose of medetomidine before general anesthesia or after detomidine in standing horses. The recovery times with or without vatinoxan also did not differ from each other. The plasma concentration of both enantiomers of medetomidine mostly correlated with the level of sedation, being lower in the presence of vatinoxan when the level of sedation was reduced.

To conclude, vatinoxan attenuated the cardiopulmonary changes and intestinal hypomotility induced by detomidine or a CRI of medetomidine in standing horses. The effects of vatinoxan on the level of sedation could be considered relatively minor. With the dosing used in this study, vatinoxan induced marked hypotension in anesthetized horses and did not influence the intestinal hypomotility associated with general anesthesia. Thus, vatinoxan represents a potential drug for alleviating or reversing the undesired effects of medetomidine and detomidine in standing horses. Its hypotensive effects, however, warrant more research before it could be usable in anesthetized horse.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their Roman numerals:

I Tapio H, Raekallio MR, Mykkänen A, Männikkö S, Scheinin M, Bennett RC, Vainio O. Effects of vatinoxan on cardiorespiratory function and gastrointestinal motility during constant-rate medetomidine infusion in standing horses. *Equine Veterinary Journal*, 2019, 51, 646-652.

II Tapio H, Raekallio MR, Mykkänen A, Al-Ramahi D, Scheinin M, Hautajärvi H, Männikkö S, Vainio O. Effects of vatinoxan on cardiorespiratory function, fecal output and plasma drug concentrations in horses anesthetized with isoflurane and infusion of medetomidine. *The Veterinary Journal*, 2019, 251:105345.

III Tapio H, Raekallio MR, Mykkänen A, Mama K, Mendez-Angulo JL, Hautajärvi H, Vainio O. Effects of MK-467 hydrochloride and hyoscine butylbromide on cardiorespiratory and gastrointestinal changes induced by detomidine hydrochloride in horses. *American Journal of Veterinary Research*, 2018, 79, 376-387.

ABBREVIATIONS

ABP	Arterial blood pressure
BBB	Blood-brain barrier
CI	Cardiac index
CNS	Central nervous system
CRI	Constant rate infusion
CVP	Central venous pressure
DAP	Diastolic arterial pressure
$\dot{D}O_2$	Oxygen delivery
$\dot{D}O_2I$	Oxygen delivery index
ET_{iso}	End-tidal isoflurane concentration
GPCR	G protein coupled receptor
HR	Heart rate
i.m.	Intramuscular
i.v.	Intravenous
LVW	Left ventricular workload of the heart
MAP	Mean arterial pressure
O_2ER	Oxygen extraction ratio
PaO_2	Arterial oxygen tension
$PaCO_2$	Arterial carbon dioxide tension
PAP	Pulmonary arterial pressure
$P_{\dot{E}}CO_2$	End-tidal carbon dioxide concentration
PIVA	Partial intravenous anesthesia
PvO_2	Venous oxygen tension
$P\bar{v}O_2$	Mixed venous oxygen tension
PVR	Pulmonary vascular resistance
PVRI	Pulmonary vascular resistance index
$\dot{Q}t$	Cardiac output
RR	Respiratory rate
SAP	Systolic arterial pressure
SV	Stroke volume
SVI	Stroke volume index
SVR	Systemic vascular resistance
SVRI	Systemic vascular resistance index
VD_{alv}:VT	Alveolar dead space ventilation
$\dot{V}O_2$	Oxygen consumption
$\dot{V}O_2I$	Oxygen consumption index

1 INTRODUCTION

Medetomidine and detomidine are α_2-adrenoceptor agonists (α_2-agonists), which are commonly used to sedate horses. In standing horses their use as CRI is particularly useful when long-lasting steady-level sedation is needed (Bettschart-Wolfensberger et al. 1999a; Wilson et al. 2002) and in anesthetized horses they have become a part of balanced general anesthesia (Bettschart-Wolfensberger and Larenza 2007). Additionally, α_2-agonists provide antinociception. Medetomidine is currently applied in off-label use for horses. However, the pharmacological characteristics of it, such as high selectivity (Virtanen et al. 1988) and short half-life in ponies (Bettschart-Wolfensberger et al. 1999a), make it an attractive choice in horses as well particularly for CRI.

α_2-agonists also produce other, often undesired and peripherally mediated effects, out of which those that influence cardiovascular and gastrointestinal function are particularly notable in horses. They increase peripheral vascular resistance and decrease the HR and $\dot{Q}t$ (Gasthuys et al. 1990; Yamashita et al. 2000). These cardiovascular events can negatively affect the $\dot{D}O_2$ to the tissues. Additionally, α_2-agonists reduce gastrointestinal motility in horses (Merritt et al. 1998; Mama et al. 2009; Elfenbein et al. 2009; Rezende et al. 2015), which is clinically relevant as horses are prone to gastrointestinal disturbances.

Vatinoxan (previously known as MK-467 or L-659,066) is an α_2-adrenoceptor antagonist that mainly acts on the peripheral α_2-adrenoceptors (Clineschmidt et al. 1988). It has potential to alleviate many of the peripheral effects of α_2-agonists without impairing their central sedative effects. Indeed, vatinoxan has been shown to diminish the cardiovascular effects of some α_2-agonists in dogs (Enouri et al. 2008; Honkavaara et al. 2011), sheep (Raekallio et al. 2010), and standing horses (Vainionpää et al. 2013), with no relevant effect on the sedation. In anesthetized horses, however, marked hypotension has been reported with the use of vatinoxan (Pakkanen et al. 2015; Wittenberg-Voges et al. 2018).

The studies included in this thesis were designed to evaluate the role of vatinoxan in diminishing the peripheral effects of medetomidine and detomidine in clinical situations in horses. The influences on cardiopulmonary and gastrointestinal function and on sedation were our main interest. In one of the studies vatinoxan was compared against hyoscine, an anticholinergic drug proposed for treating decreased HR in horses (Pimenta et al. 2011). Additionally, we evaluated the drug concentrations in plasma to facilitate our understanding of their clinical responses. We hypothesized that adding vatinoxan in the premedication before a medetomidine CRI would alleviate the cardiopulmonary changes characteristic of this α_2-agonist in standing and anesthetized horses without a relevant influence on sedation (studies I and II). Furthermore, we anticipated that vatinoxan would improve the decreased intestinal motility associated with a medetomidine CRI and general anesthesia (studies I–II). Finally, we expected vatinoxan to reverse the cardiopulmonary and intestinal effects of detomidine without influencing the level of sedation (study III).

2 REVIEW OF THE LITERATURE

2.1 Blood-brain barrier

Besides bony structures, meninges, and cerebro-spinal fluid, the CNS is also protected by the BBB. This barrier essentially separates the interstitial fluid of the CNS from other circulation (see review in Dyrna et al. 2013). The main functions of the BBB are to create a stable environment for the CNS and to protect it from the neuroexcitatory substances of plasma and from neurotoxins (Dyrna et al. 2013). Tight junctions between capillary endothelial cells are a crucial part of the BBB in the vertebrate brain, although other related cells are also important (Brightman and Reese 1969). It is of notice that the BBB is anatomically and functionally different from the blood–cerebrospinal fluid barrier—for example, a substance entering the cerebrospinal fluid will therefore not necessarily access the brain (review in Pardridge 2012). Substances may enter the brain through a passive diffusion across the BBB, or they may require active transport systems (Dyrna et al. 2013). The movement of a substance across the BBB can be regulated by the BBB itself and also by the characteristics of the substance and the cellular environment (such as pH) (Huwyler et al. 1997; Dyrna et al. 2013).

2.2 Adrenoceptors

Adrenergic receptors (adrenoceptors) mediate the actions of the sympathetic nervous system that is crucial for the maintenance of physiological body functions and influences virtually every tissue in some way (see review in Wehrwein et al. 2016). The term adrenergic refers to the main endogenous neurotransmitter of these receptors; noradrenaline, that is considered the main postganglionic neurotransmitter of the sympathetic nervous system. Several studies have later revealed that many other substances that may act as neurotransmitters or modulators are also abundant in the autonomic nervous system (see reviews in Burnstock 2009; Wehrwein et al. 2016).

Adrenoceptors belong to the large family of GCPR (see review in Schwinn 1993). All GPCR, and thus also adrenoceptors, are excitable transmembrane proteins with a seven-loop alpha-helical structure and an extracellular amino terminus and intracellular carboxyl terminus (review by Rosenbaum et al. 2009). A ligand (such as noradrenaline) binding to the receptor allows the GPCR to couple with a specific G protein residing in the cell membranes. The G protein recognizes the activated GPCR and is consequently able to activate various intracellular pathways that eventually result in the biological response of the cell (Schwinn et al. 1993; Rosenbaum et al. 2009).

2.2.1 α_2-adrenoceptors

Adrenoceptors are divided into three groups—α_1, α_2, and β—each of which has further three subtypes (see review in Schwinn 1993; Bylund et al. 1994). α_1- and α_2-adrenoreceptors are expressed in many organs and systems of mammals (Ruffolo and Hieble 1994), but the expression of the adrenoceptors may vary across species. For example, α_2-adrenoceptors have not been detected in equine platelets, whereas they are expressed in the platelets of cats and dogs (Hikasa et al. 2013).

On a cellular level, both pre- and postganglionic α_2-adrenoceptors exist (Van Zwieten and Timmermans 1984). Preganglionic α_2-receptors control the release of a neurotransmitter from the nerve ends, i.e. a ligand binding to these receptors inhibits the release of noradrenaline. This is called autoregulation. Postganglionic α_2-adrenoceptors transmit neural signals to the target organs. Furthermore, extrasynaptic receptors, located at a distance from the nerve endings, also exist in, for example, the vascular smooth muscle of rats (Wilffert et al. 1982).

2.2.2 Subtypes of α_2-adrenoceptors

The subtypes of α_2-adrenoceptors are coded by three genes and are known as α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors (Bylund et al. 1994; Philipp et al. 2002; Knaus et al. 2007). There is yet another subtype, α_{2D}, which is coded by the same gene as α_{2A} but differs from it pharmacologically across different species (Bylund et al. 1994; Guimarães and Moura 2001).

All three subtypes are spread widely in the CNS of mice and rats (Scheinin et al. 1994; Sallinen et al. 1997) and involved in mediating antinociception (see the review of α_2-adrenoceptor subtypes in mice in Philipp et al. 2002). The sedative effects are mediated solely by preganglionic central α_{2A}-adrenoceptors located mainly in the *locus coeruleus* of the brain (Philipp et al. 2002).

With respect to the peripheral clinical effects mediated by α_2-adrenoceptors, the α_{2A} and α_{2B} subtypes are mainly responsible for the biphasic vascular effects, but they function in opposite ways. Central α_{2A}-adrenoceptors mediate vasodilation (MacMillan et al. 1996), and the peripheral α_{2B}-receptors at the vessel walls are responsible for vasoconstriction (Link et al. 1996). A study conducted with knock-out mice and medetomidine revealed that the α_2-adrenoceptor subtype α_{2A} is likely responsible for the intestinal motility–inhibiting effect in mice (Scheibner et al. 2002). Furthermore, in guinea pigs, the same subtype α_{2A} was reported to modulate the release of acetycholine by cholinergic nerve fibers in Auerbach´s plexus (Blandizzi et al. 1991).

2.3 α_2-adrenoceptor agonists

α_2-agonists are drugs that activate α_2-adrenoceptors. Their main indication in veterinary medicine is sedation and antinociception, and they were first reported for this purpose in the 1960s (Clarke and Hall 1969). Dexmedetomidine, medetomidine detomidine, romifidine, and xylazine are α_2-agonists that are labeled for veterinary use. Another α_2-agonist, clonidine, available for humans for the treatment of hypertension, has been administered experimentally for horses but is not in clinical use (Dirikolu et al. 2006).

2.3.1 α_2-adrenoceptor selectivity

α_2-agonists also have an affinity for α_1-adrenoceptors, but the affinity ratio for these two adrenoceptors (α_1 and α_2) differs between these drugs. Medetomidine and its active enantiomer dexmedetomidine are the most selective α_2-agonists, with a selectivity ratio (α_2:α_1) of 1620:1 (Virtanen et al. 1988). The reported selectivity ratios for xylazine, detomidine and clonidine are 160:1, 260:1 and 220:1, respectively (Virtanen et al. 1988). Selectivity ratio of 340:1 has been quoted for romifidine (see review in Gozalo-Marcilla et al. 2015). High α_2-selectivity is preferable as it translates into a higher specificity and potency of the drug. α_1-receptors can contribute to the undesired effects in the cardiovascular and gastrointestinal systems of a horse, as they mediate the contraction of vascular smooth muscle (Guimarães and Moura 2001) and the relaxation of intestinal smooth muscle (Rang et al. 2016a). Furthermore, α_1-agonists have been shown to have local thermal hyperalgesic effects (Dogrul et al. 2006) and potentially impair the analgesic effects mediated via α_2-receptors in mice (Gil et al. 2009). Detomidine, medetomidine, or xylazine have not been shown to display higher selectivity for any α_2-adrenoceptor subtype over another (Schwartz and Clark 1998).

2.3.2 Imidazoline receptors

Imidazole derivatives (such as detomidine and medetomidine) can also bind to imidazoline receptors (see review in Ernsberger et al. 1997). Thus, some of their effects may be mediated through these receptors, albeit the role of imidazoline receptors is not clear yet (Li and Zhang 2011). To date, three imidazoline receptor subtypes have been proposed (I_1, I_2, and I_3), and they are involved in regulating blood pressure centrally (I_1), in insulin release from pancreatic cells (I_3), and in many neurobiological events, such as depression and analgesia (I_2) (Head and Mayorov 2006; Li and Zhang 2011). The available knowledge about the affinity of clinically used α_2-agonists for imidazoline receptors is scarce. Very low selectivity has been reported for detomidine and medetomidine at least in the case of the I_1-receptor in bovine tissue (Ernsberger et al. 1997).

2.3.3 Sedative and antinociceptive effects

Sedative and antinociceptive effects of α_2-agonists are attributed to receptors at the *locus coeruleus*, as it is a main noradrenergic nucleus located at the pons and is an important part in regulating, for example, sleep, arousal, nociception, as well as cognitive functions (see review in Gertler et al. 2001). The anesthetic properties are proposed to occur via the enhancement of potassium conductance and the hyperpolarization of cells, resulting in decreased neuronal firing (Scheinin and MacDonald 1989; Gertler et al. 2001). Additionally, the entry of calcium into the cell is inhibited, which may further suppress the release of neurotransmitters (Gertler et al. 2001).

Nociception may be modulated in the CNS by α_2-agonists on supraspinal and spinal levels. α_2-agonists are thought to suppress the pain signals via the autoregulation of α_2-adrenoreceptors and thus prevent the pain signal from propagating (Gertler et al. 2001). α_2-adrenoceptors are expressed in areas of the brain that participate in nociception, such as the *locus coeruleus* and midbrain regions (Scheinin et al. 1994). Furthermore, when injected into the subarachnoidal space of the spinal cord, α_2-agonists produce profound antinociception in several species (see review in Yaksh 1985).

2.3.4 Cardiopulmonary effects

The autonomic nervous system is of major importance in the control of the cardiovascular system. α_2-agonists affect the cardiovascular system through both central and peripheral adrenoceptors. The effect of α_2-agonists on blood pressure is biphasic. Initially, the activation of the peripheral α_2-adrenoceptors, particularly of subtype α_{2B} (Philipp et al. 2002), on the walls of blood vessels causes vasoconstriction (Flacke et al. 1990). As a consequence, SVR increases and ABP rises (Flacke et al. 1990). The second phase of the blood pressure changes is mainly centrally mediated. When the central α_2-adrenoceptors are activated, sedation is induced and the central sympathetic outflow decreases (Virtanen 1986). As a result of decreased sympathetic outflow, ABP decreases. Several regions of the brain, particularly the rostroventrolateral medulla (the pressor area of the medulla), have been attributed to the hypotensive effects of α_2-agonists (Fornai et al. 1990; Samuels and Szabadi 2008).

Bradycardia and decreased $\dot{Q}t$ are further typical cardiovascular effects of α_2-agonists. Bradycardia is thought to ensue after the increase in SVR mainly as a result of the baroreflex. Flacke et al. (1992) found dexmedetomidine to decrease both $\dot{Q}t$ and SV in dogs, but it did not have a direct effect on the contractility of the myocardium. Therefore, the authors concluded that the clinically observed suppression in cardiac performance is likely exerted by other, indirect mechanisms. However, at least the human right atrium (review in Brodde et al. 2001) and rat cardiomyocytes (Maltsev et al. 2014) have been shown to express α_2-adrenoceptors, and in a functional *in vitro* study, the presence of α_2-adrenoceptors in the equine aortic valve was suggested (Bowen et al. 2004). Finally, dexmedetomidine been shown to reduce myocardial energy requirements (Lawrence et al. 1996) and to maintain blood flow to ischemic myocardial regions experimentally in dogs (Roekaerts et al. 1996).

In conscious dogs, the effect of dexmedetomidine on respiratory drive was mild (Nguyen et al. 1992). However, ventilatory response to hypercapnia may be diminished in dogs receiving dexmedetomidine i.v. (Nguyen et al. 1992; Sabbe et al. 1994). In rabbits, respiratory depression with reduced response to hypercapnia was also observed but no profound hypoxemia or hypercapnia was induced (Nishida et al. 2002).

2.3.5 Gastrointestinal effects

The CNS controls the gastrointestinal function via the sympathetic and the parasympathetic system (see review in Browning and Travagli 2014). Additionally, the intrinsic enteric nervous system also plays an important role in modulating gastrointestinal functions (see reviews in Furness 2012 and in Browning and Travagli 2014).

The data on the distribution of α_2-adrenoreceptors and their function in the gastrointestinal tract is largely based on studies on species other than horses (De Ponti et al. 1996). Both pre- and postsynaptic α_2-adrenoceptors are present in the entire gastrointestinal tract from the esophagus to the large colon. Postsynaptic α_2-adrenoreceptors located in the smooth muscle of the intestine primarily mediate the inhibition of gastrointestinal motility (De Ponti et al. 1996). Besides smooth muscle, α_2-adrenoreceptors are also found in enterocytes, where they promote water and electrolyte intake (De Ponti et al. 1996). Additionally, there is evidence that the cholinergic motor activity of the gastrointestinal tract can be modulated by presynaptic α_2-adrenoreceptors at least in guinea pigs (Blandizzi et al. 1991; Colucci et al. 1998).

Generally, α_2-agonists inhibit gastrointestinal motility. In dogs, medetomidine has been shown to inhibit the contractile activity of the small intestine and colon, and this action was attributed to peripheral α_2-adrenoceptors (Maugeri et al. 1994). The inhibiting effect of the colon was preceded by a short (10–20 min) excitatory effect on contractility. In rats, clonidine induced the inhibition of small intestine contractions, but it was followed by an irregular spiking activity (Fargeas et al. 1986). The first effect (inhibition) was attributed, in part, to peripheral α_2-adrenoceptors and the latter to central receptors.

In an *in vitro* study with equine small intestine preparations, α_2-agonists decreased the contractility of the intestine (Zullian et al. 2011). Conversely, in other *in vitro* studies with horses (Malone et al. 1996) and dogs (Zhang et al. 1992), intestinal smooth muscle contractility actually increased with α_2-agonists. In horse esophagus preparations *in vitro*, detomidine had no effect on smooth muscle (or skeletal muscle) contractility, and its relaxing effects on the esophagus were thus postulated to be central (Wooldridge et al. 2002).

2.4 Detomidine and (dex)medetomidine in horses

Detomidine, xylazine and romifidine are α_2-agonists registered for veterinary use in horses. Dexmedetomidine and medetomidine are used off-label as they are currently registered only

for small animals and not included in the drugs allowed for food-producing animals (European Union legislation, directive 37/2010).

The clinical effects of α_2-agonists are dose-dependent in horses (Jöchle and Hamm 1986; Bryant et al. 1996; Yamashita et al. 2000) as has been also observed in other species, such as dogs (Pypendop and Verstegen 1998). Additionally, it should be noted that nervous horses that have a higher pre-sedation plasma concentration of catecholamines are less-responsive to the sedative effect of α_2-agonists compared to calmer horses (Raekallio et al. 1992).

2.4.1 Pharmacokinetics

α_2-agonists are mainly administered parenterally and most commonly via the i.v. route in horses. An i.m. administration route is occasionally used, and detomidine is also available as a sublingually administrable gel.

The maximum concentration of detomidine in plasma after i.m. administration has been reported to occur at 30–70 min with doses of 30–80 µg/kg (Salonen et al. 1989; Grimsrud et al. 2009), being faster with a higher dose. In comparison, the maximum concentration of detomidine administered sublingually on the oral mucosa (40 µg/kg) was reached at 1.8 h when the blood samples were collected from the thoracic vein (Kaukinen et al. 2011), but the time was reduced to only 36 min when the samples were obtained from the jugular vein, into which the oral mucosa drains (DiMaio Knych et al. 2011). The reported bioavailability of i.m.-administered detomidine is 54% ± 14% (Grimsrud et al. 2009) or 38% ± 8% (Kaukinen et al. 2011), depending on the study. In the study by Kaukinen et al. (2011), the bioavailability of sublingually administered detomidine was 22% ± 5%. In another study, higher plasma concentrations of sublingually administered detomidine were obtained than in the study by Kaukinen et al. (2011), but the bioavailability was not calculated (DiMaio Knych et al. 2011). The free fraction of detomidine in plasma has been reported to be 8%–15% in dogs, calves, and horses (Salonen 1986). Detomidine is metabolized in the liver and mainly excreted in urine (Salonen 1986; Salonen et al. 1989). With clinically relevant, single doses (30–40 µg/kg) in horses, the elimination half-life of detomidine has been found to be approximately 30–50 min from i.v. administration (Grimsrud et al. 2009; Kaukinen et al. 2011), 1 h from i.m. administration (Grimsrud et al. 2009; Kaukinen et al. 2011), and 1.3–1.5 h from sublingual administration (Kaukinen et al. 2011; DiMaio Knych et al. 2011).

The reported elimination half-life of medetomidine (7–10 µg/kg i.v.) is 11–29 min in ponies (Bettschart-Wolfensberger et al. 1999a) and horses (Grimsrud et al. 2012) and that of dexmedetomidine (3.5 µg/kg i.v.) 20–29 min in ponies (Bettschart-Wolfensberger et al. 2005). In the study by Bettchart-Wolfensberger et al. from 2005, the elimination half-life of dexmedetomidine was slower (29 min) in older ponies. In humans, an average of 94% of dexmedetomidine is bound to plasma proteins (Gertler et al. 2001). Similarly to detomidine, medetomidine is mainly metabolized in the liver by means of direct glucuronidation and hydroxylation and excreted in urine in rats (Salonen and Eloranta 1990) and humans (Gertler et al. 2001). However, the reported clearance of dexmedetomidine in horses is higher than the

blood flow of the liver, which is why the possibility of an extrahepatic metabolism of dexmedetomidine has been speculated (Rezende et al. 2015). Furthermore, due to the changes that these drugs induce in the cardiovascular system, they are likely affecting their own disposition, as has been reported in cats (Pypendop et al. 2013) and humans (Dutta et al. 2000). Indeed, detomidine was recently reported to decrease its own clearance in a concentration-dependent fashion also in horses (Gozalo-Marcilla et al. 2019a). Finally, in conscious horses receiving dexmedetomidine as CRI, considerable variation was detected in drug concentrations in plasma (Ranheim et al. 2015). However, in isoflurane-anesthetized horses receiving dexmedetomidine CRI the dexmedetomidine concentration in plasma was more stable (Risberg et al 2016; Bettembourg et al. 2019).

2.4.2 Sedative and antinociceptive effects in horses

Detomidine is widely used in equine practice to sedate horses for various procedures. It has already been available for over 30 years (Clarke and Taylor 1986; Jöchle and Hamm 1986; Lowe and Hilfiger 1986; Virtanen 1986). Several studies have described its use as single or repeated i.v. boluses in horses for sedation and antinociception (see, for example, the review by England and Clarke 1996; Moens et al. 2003; Mama et al. 2009). Initially, the described doses were high, up to 160 µg/kg i.v. (Jöchle and Hamm 1986), but single bolus doses up to 30 µg/kg i.v. are currently commonly used (Wagner et al. 1991; Mama et al. 2009; Gozalo-Marcilla et al. 2017). Furthermore, detomidine has also been successfully used as a CRI in clinical cases sedated for various surgical procedures (Wilson et al. 2002; Potter et al. 2016). The reported initial rate of CRI was 0.6 µg/kg/min, which was then halved every 15 min (Wilson et al. 2002) or adjusted to effect (Potter et al. 2016).

In a study with thoroughbred horses, medetomidine (7.5 µg/kg i.v.) provided sufficient sedation with only mild ataxia when the horses were stimulated by, for example, touching their heads and feet and producing sounds (Hobo et al. 1995). With a higher dose (10 µg/kg i.v.), the level of sedation did not increase, but the ataxia worsened (Hobo et al. 1995). Initially, a dexmedetomidine dose of 3.5 µg/kg i.v. has been proposed for horses (Bettschart-Wolfensberger et al. 2005), but later studies have reported that in clinical cases it may not produce sufficient sedation (Marcilla et al. 2012; Marly-Voquer et al. 2016). In another study, dexmedetomidine (5 µg/kg i.v.) produced sedative and antinociceptive effects in healthy horses for roughly 1 h and 30 min, respectively (Rezende et al. 2015). The most severe ataxia was observed during the first 15 min after (dex)medetomidine administration (Hobo et al. 1995; Rezende et al. 2015).

A medetomidine loading dose of 5 µg/kg i.v. and a CRI of 3.5 µg/kg/h has been found to provide a constant level of sedation in ponies that were not stimulated (Bettschart-Wolfensberger et al. 1999a). With dexmedetomidine, the sedative and antinociceptive effects have been observed at CRI rates of 2–8 µg/kg/h (Risberg et al. 2014; Ranheim et al. 2015), although at the rate of 2 µg/kg/h the antinociception was suspected to be weak (Risberg et al. 2014). The level of sedation paralleled with the plasma drug concentrations, and the authors postulated that the sedative response is easily adjustable with changing the CRI rate also in

clinical cases (Ranheim et al. 2015). However, there may be large individual variation in concentration of dexmedetomidine in plasma (Risberg et al. 2014; Ranheim et al. 2015). In clinical cases, a medetomidine CRI (loading dose 5 µg/kg i.v. and CRI 5 µg/kg/h) combined with a CRI of morphine has been successfully used for laparoscopic surgery in thoroughbreds (Solano et al. 2009). Additional medetomidine i.v. boluses, however, were required in some horses while the laparoscopic portal sites were injected with local anesthetics. Furthermore, dexmedetomidine CRI has been used to sedate standing horses for instrumentation in transvenous electrical cardioversion (for atrial fibrillation) and as an adjunct to isoflurane to anesthetize horses for cardioversion treatment (Marly-Voquer et al. 2016).

α_2-agonists can be combined with other drugs, chiefly opioids, in order to reduce the possible hypersensitivity to stimulus, such as touch, associated with α2-agonists alone (see review in England and Clarke 1996) and in order to improve perioperative analgesia (Solano et al. 2009; Potter et al. 2016; Gozalo-Marcilla et al. 2019b). Finally, in horses, α_2-agonists are administered for antinociception mainly i.v., but the epidural and subarachnoid routes can also be used to achieve caudal analgesia (see review in Daunt and Steffey 2002).

2.4.3 Cardiopulmonary effects in horses

α_2-agonists produce their typical cardiopulmonary effects in horses as well. After detomidine administration (10–40 µg/kg i.v.) MAP of 160–170 mmHg (Gasthuys et al. 1990; Yamashita et al. 2000) and HR of 19–33 beats/min have been reported within minutes after administration (Gasthuys et al. 1990; Yamashita et al. 2000; Mama et al. 2009). In comparison, after the administration of medetomidine (5–10 µg/kg i.v.), MAP of 120–138 mmHg (Yamashita et al. 2000) and HR of 25–30 beats/min (Yamashita et al. 2000; Grimsrud et al. 2012) were observed. In addition to bradycardia, conductance disturbances, such as 2nd degree atrioventricular blocks of the heart, are observed in horses with α_2-agonists (Gasthuys et al. 1990; Bettschart-Wolfensberger et al. 1999b; Yamashita et al. 2000). These acute responses, hypertension and bradycardia, to medetomidine have been attributed to the activation of α_2-receptors rather than α_1-receptors in horses (Bryant et al. 1998).

A hypotensive phase after the initial hypertension has been observed with single i.v. doses of detomidine (10–20 µg/kg) in horses (Wagner et al. 1991) and medetomidine (5–10 µg/kg) in ponies (Bryant et al. 1996). Conversely, with higher i.v. doses of detomidine (20 and 40 µg/kg), the hypertensive phase has been prolonged and not followed by hypotension, at least during the observation period of 120 min (Yamashita et al. 2000). In response to the reduced sympathetic tone, the HR stabilizes after the initial hypertensive phase but tends to remain decreased with detomidine (Gasthuys et al. 1991; Yamashita et al. 2000) and medetomidine (Bettschart-Wolfensberger et al. 1999b; Yamashita et al. 2000).

Decreased $\dot{Q}t$ has also been observed with dexmedetomidine (Bettschart-Wolfensberger et al. 2005), medetomidine (Yamashita et al. 2000), and detomidine (Gasthuys et al. 1990; Daunt et al. 1993; Yamashita et al. 2000; Pimenta et al. 2011). Some of these studies report decreases in SV with dexmedetomidine (Bettschart-Wolfensberger et al. 2005),

medetomidine, and detomidine (Yamashita et al. 2000), but others have detected no changes in it after similar doses of detomidine (Gasthuys et al. 1990; Pimenta et al. 2011). Buhl et al. (2007) demonstrated in horses that detomidine and, to a lesser extent, romifidine altered the echocardiographic measurements of the heart, which indicated a reduced SV after the administration of these two α_2-agonists. Furthermore, valvular regurgitation was increased after detomidine and romifidine administration, which was attributed to increased afterload and bradycardia (Buhl et al. 2007).

A transient increase in PAP has been observed in standing horses after a bolus dose of detomidine (20–40 μg/kg i.v.) (Wagner et al. 1991; Yamashita et al. 2000; Nyman et al. 2009), medetomidine (7.5–10 μg/kg i.v.) (Yamashita et al. 2000), dexmedetomidine (3.5 μg/kg i.v.) (Bettschart-Wolfensberger et al. 2005) and romifidine (80 μg/kg i.v.) (Ringer et al. 2013). Similarly to systemic ABP, the effect of detomidine on PAP was biphasic in one report, and the PAP decreased transiently after 45–60 min (Wagner et al. 1991). With a CRI of medetomidine (5 μg/kg loading dose followed by 3.5 μg/kg/h CRI i.v.), however, no changes in PAP were detected (Bettschart-Wolfensberger et al. 1999b). Reports about the effects of α_2-agonists on PVR in a standing horse are scarce. An increase in PVR, along with PAP, after a single dose of detomidine was reported (Wagner et al. 1991; Nyman et al. 2009) and during a romifidine CRI suspected because of increased PAP (Ringer et al. 2013) in horses, whereas a CRI of medetomidine has been reported to induce no detectable changes in PVR (nor in PAP) in ponies (Bettschart-Wolfensberger et al. 1999b).

Reports on the RR and arterial blood gases in horses receiving α_2-agonists are somewhat controversial. A decreased RR was observed after administration of detomidine (10–40 μg/kg i.v.) (Yamashita et al. 2000), medetomidine (5–10 μg/kg i.v.) (Yamashita et al. 2000), and dexmedetomidine (3.5 μg/kg i.v.) (Bettschart-Wolfensberger et al. 2005) in some studies, while others reported no changes in RR with a similar dosing of detomidine (Reitemeyer et al. 1986; Nyman et al. 2009). Furthermore, Nyman et al. (2009) did not observe changes in minute ventilation or tidal volume after detomidine sedation, although $PaCO_2$ increased, suggesting slight hypoventilation. In contrast, Wagner et al. (1991) detected no changes in $PaCO_2$ despite a decreased RR. Decreases in PaO_2 have been reported with detomidine (Gasthuys et al. 1990; Yamashita et al. 2000; Nyman et al. 2009) and medetomidine (Yamashita et al. 2000), although others using similar doses did not observe the decrease (Bryant et al. 1996; Pimenta et al. 2011). Mixed venous O_2 tension is reported in fewer studies on detomidine or medetomidine in horses, but it has been found to decrease after a single dose of detomidine (Nyman et al. 2009), a detomidine infusion (Daunt et al. 1993), and transiently 5 min after a medetomidine loading dose given before a medetomidine CRI (Bettschart-Wolfensberger et al. 1999b).

2.4.4 Gastrointestinal effects in horses

Gastrointestinal borborygmi, assessed by auscultation, has been reported to decrease after the administration of detomidine (Mama et al. 2009; Pimenta et al. 2011; Vainionpää et al. 2013; Gozalo-Marcilla et al. 2017; 2019b), medetomidine (Grimsrud et al. 2012), and

dexmedetomidine (Rezende et al. 2015). The decrease in borborygmi was faster and of greater magnitude when detomidine was administered i.v. compared to i.m. administration (Mama et al. 2009). The intestinal transit time, however, was not influenced by detomidine (Pimenta et al. 2011), although defecation was subjectively assessed to be reduced during the first hours after sedation with both detomidine (Mama et al. 2009) and medetomidine (Grimsrud et al. 2012).

Detomidine has also been shown to decrease duodenal motility (Merritt et al. 1998; Elfenbein et al. 2009), and medetomidine has been demonstrated to decrease jejunal, cecal, and colonic contractility (Sasaki et al. 2000). Furthermore, Sasaki et al. (2000) suggested that the jejunum may be less sensitive to the effects of α_2-agonists than the cecum or ventral colon. Similarly to dogs (Maugeri et al. 1994), a short period of increased tone preceded the inhibiting pattern on colon motility in ponies after detomidine administration (Roger and Ruckebusch 1987). Additionally, detomidine delayed the gastric emptying rate in a dose-dependent manner in healthy horses (Sutton et al. 2002).

While α_2-agonists decrease motility in the equine gastrointestinal tract, they can also be beneficial in the treatment of acute colic. α_2-agonists have been shown to alleviate visceral pain in a colic model, as has been shown with detomidine (Lowe and Hilfiger 1986; Roger and Ruckebusch 1987; Elfenbein et al. 2009) and xylazine (Lowe and Hilfiger 1986; Roger and Ruckebusch 1987). Roger and Ruckebusch (1987) simulated intestinal hypermotility and colic with an infusion of prostaglandins, and detomidine (60 μg/kg i.v.) relaxed the entire large intestine for over 1 h and alleviated colic signs for over 2 h in ponies. The increase in nociceptive threshold with cecal or colorectal distension was dose-dependent and lasted up to 165 min with detomidine (20 μg/kg i.v.) (Lowe and Hilfiger 1986; Elfenbein et al. 2009), whereas significant increase in antinociception for duodenal distention was noted only at 15 min (Elfenbein et al. 2009). Lowe and Hilfiger (1986) suggested that relaxing the intestinal smooth muscle may contribute to visceral analgesia in a distention colic model.

2.5 α_2-agonists and general anesthesia in horses

Equine anesthesia carries a relatively high risk for the animal, with the reported mortality rates of 0.9% for healthy horses in a large multi-center study (Johnston et al. 2002) and 0.12%–1.1% for all cases in studies with smaller sample sizes (Bidwell et al. 2007; Dugdale et al. 2016). The main concerns during equine anesthesia are the depression of the cardiopulmonary function and hence tissue perfusion and oxygenation, as well as recovery from the anesthesia. The improved cardiovascular monitoring and support have reduced fatalities during the anesthesia itself, but some of the horses are still lost because of catastrophes during the recovery phase (see review in Dugdale and Taylor 2016). The success of equine anesthesia is affected by several factors related to the physical condition and age of the horse, the type and timing of surgery, the duration of anesthesia, the drugs administered, and the facilities and personnel involved (Johnston et al. 2002; Dugdale and Taylor 2016).

2.5.1 Changes in cardiopulmonary function during general anesthesia

General anesthesia markedly alters the cardiopulmonary function in horses. The altered function can be mainly considered to result from the position of the horse and the effects of anesthetic drugs.

Recumbency causes mechanical pressure on the dependent muscles, which can impair the blood flow in these regions. The maintenance of a MAP of over 70 mmHg in an anesthetized horse has been suggested as a limit for maintaining muscle perfusion and reducing the severity of post-anesthetic myopathy (Lindsay et al. 1989; Young and Taylor 1993). Furthermore, the atelectasis of the dependent lung occurs soon after recumbency in anesthetized horses, leading to a ventilation–perfusion mismatch (Nyman et al. 1990). Impaired lung function can impair oxygenation, and, indeed, dorsal or lateral recumbency has been reported by several authors to induce hypoxemia in horses (see review in Auckburally and Nyman 2017). While hypoxemia can be life-threating, less severe but nevertheless important complications may also ensue. For example, a PaO_2 of less than 80 mmHg during anesthesia, in addition to certain suture materials and anesthesia duration, have been found to increase the risk of surgical site infection after exploratory laparotomy (Costa-Farré et al. 2014). Lastly, dorsal recumbency may reduce venous return more than lateral recumbency, as was observed in a study during halothane anesthesia (Stegmann and Littlejohn 1987).

Volatile anesthetic agents, such as isoflurane and sevoflurane, have a cumulative, suppressive effect on cardiovascular function, and both of these agents are also respiratory depressants (Grosenbaugh and Muir 1998). Isoflurane has been demonstrated to decrease the ABP and tended to decrease SVR and $\dot{Q}t$, although the role of lateral recumbency could not be differentiated (Steffey et al. 1987). The hemodynamic effects of sevoflurane have been shown resemble those of isoflurane (Grosenbaugh and Muir 1998). The use of sedative premedication prior to inducing general anesthesia, and particularly the use of acepromazine, has been associated with a reduced mortality rate with equine anesthesia (Johnston et al. 2002). This was attributed to decreases in circulating catecholamines and, consequently, the horse requiring less anesthetics. Interestingly, premedication with romifidine, as opposed to other α_2-agonists, tended to increase mortality in the study by Johnston et al. (2002).

2.5.2 Medetomidine and dexmedetomidine infusion during general anesthesia

Balanced, or multimodal, general anesthesia refers to co-administration of different drugs in order to calm the patient, and reduce pain and the likelihood of potential adverse effects of a single drug (Bettschart-Wolfensberger and Larenza 2007). It is often achieved in horses by PIVA, thus administering i.v. agents such as α_2-agonists together with volatile agents for anesthesia (see review in Bettschart-Wolfernsberger and Larenza 2007; Gozalo-Marcilla et al. 2014; 2015). In horses the main aims of the balanced anesthesia are the maintenance of

adequate cardiopulmonary function and achievement of good quality recovery (Bettschart-Wolfensberger and Larenza 2007).

Medetomidine CRI as part of PIVA has been reported to provide reliable anesthesia for various types of elective surgery, such as arthroscopies, fracture repairs, or urogenital procedures (Kalchofner et al. 2006). Furthermore, CRIs of medetomidine (Bettschart-Wolfensberger et al. 2001; Ringer et al. 2007), dexmedetomidine (Gozalo-Marcilla et al. 2013a) , detomidine, and romifidine (Niimura Del Barrio et al. 2017) have been reported to reduce the amount of inhalation agent, such as isoflurane or desflurane, used during anesthesia in horses. This anesthetic sparing effect could not be confirmed in some studies (Devisscher et al. 2010; Schauvliege et al. 2011; Marcilla et al. 2012), potentially due to subjective evaluation and adjustment of the depth of the anesthesia (Marcilla et al. 2012). Horses may appear clinically more lightly anesthetized during isoflurane anesthesia when receiving medetomidine CRI (Ringer et al. 2007). However, adjunctive dexmedetomidine CRI (Gozalo-Marcilla et al. 2013b) and medetomidine CRI (Ringer et al. 2007) have been reported to produce more easily stable anesthetic depth in isoflurane-anesthetized horses than morphine (Gozalo-Marcilla et al. 2013b) or lidocaine (Ringer et al. 2007) CRI.

A (dex)medetomidine CRI during anesthesia has been shown to prolong and improve the quality of recovery compared to no adjunctive CRI (Marcilla et al. 2012) or lidocaine CRI (Ringer et al. 2007), judged as fewer necessary attempts to stand up and the horse showing less ataxia while recovering from anesthesia. Furthermore, better recoveries were associated with an adjunctive dexmedetomidine CRI than with a medetomidine CRI during isoflurane anesthesia, although the authors of the study questioned the comparability of the recovery results due to more additional doses administered for the dexmedetomidine group (Sacks et al. 2017).

The characteristic cardiovascular effects are a concern in using a CRI of (dex)medetomidine during anesthesia, although controversy exists as to the significance of these effects. The CI has been shown to decrease and the O_2ER to increase, when an adjunctive dexmedetomidine CRI (1.75 µg/kg/h) is added to isoflurane anesthesia (Risberg et al. 2016). A decreased HR and CI have also been reported in other studies with (dex)medetomidine during isoflurane anesthesia, but these changes have been judged limited (Ringer et al. 2007; Marcilla et al. 2010; 2012; Bettembourg et al. 2019). Mean arterial pressure, in turn, was better maintained when an adjunctive dexmedetomidine CRI was administered during isoflurane anesthesia (Risberg et al. 2016), although this effect could not be detected in another study (Marcilla et al. 2012). When comparing CRIs of dexmedetomidine and medetomidine with other adjunctive CRIs—morphine (Gozalo-Marcilla et al. 2013b), lidocaine (Ringer et al. 2007), and S-ketamine (Menzies et al. 2016)—during isoflurane anesthesia, the CI was consistently lower with a CRI of (dex)medetomidine, and the MAP was either higher (Ringer et al. 2007; Gozalo-Marcilla et al. 2013b) or no difference could be detected (Menzies et al. 2016).

The rate of medetomidine CRI commonly reported for PIVA is the same (3.5 µg/kg/h) that produced stable sedation in conscious ponies (Bettschart-Wolfensberger et al. 1999b).

The rate of dexmedetomidine CRI has been extrapolated from that (Marcilla et al. 2010) considering that medetomidine is a racemic mixture containing 50% of active enantiomer, dexmedetomidine. No differences were detected in cardiopulmonary function between medetomidine (3.5 µg/kg/h) and dexmedetomidine (1.75 µg/kg/h) in a study comparing CRIs of the two α_2-agonists during isoflurane anesthesia in horses (Sacks et al. 2017). Moreover, no differences in cardiopulmonary function were reported in two other studies between two different dose rates of dexmedetomidine CRI (1 and 1.75 µg/kg/h) during isoflurane anesthesia in horses (Marcilla et al. 2010; Bettembourg et al. 2019) albeit the authors of the latter study acknowledged that the cardiopulmonary monitoring was limited (Bettembourg et al. 2019).

2.5.3 Gastrointestinal function and general anesthesia

Post-anesthetic colic is a well-recognized phenomenon in horses, with a mean prevalence of up to 8.7%, depending on the study and the criteria for gastrointestinal dysfunction (Senior et al. 2006; Nelson et al. 2013). In one multicenter report, the most common type of colic was large intestinal impaction, and the horses anesthetized for orthopedic surgery were at an increased risk (Senior et al. 2006). There is doubt whether perianesthetic drugs, particularly morphine, increase the risk for post-anesthetic colic, but α_2-agonists have not been identified as a risk-increasing factor (Senior et al. 2006; Andersen et al. 2006; Nelson et al. 2013). Several other factors, such as increased blood lactate levels during anesthesia and delayed fecal output, have been found to be associated with post-anesthetic gastrointestinal dysfunction (Nelson et al. 2013). An increased lactate level was attributed to increased anaerobic metabolism, which can result from inadequate tissue perfusion or oxygenation (Nelson et al. 2013). Furthermore, the maintenance of adequate cardiovascular status during anesthesia also influences the perfusion of the gastrointestinal tract. During isoflurane anesthesia, the microperfusion of the jejunum and colon significantly decreased, when the $\dot{Q}t$ decreased to below 50 mL/kg/min and the MAP to below 60 mmHg (Hopster et al. 2015).

2.6 α_2-adrenoceptor antagonists

In small animals, the sedation induced by α_2-agonists is routinely reversed by α_2-adrenoceptor antagonists (α_2-antagonists). In horses, α_2-antagonists are not in routine clinical use. Currently, there are no α_2-antagonists registered for veterinary use in horses, but in experimental studies their effects have been described in horses as well.

2.6.1 Atipamezole, tolazoline, and yohimbine in horses

Atipamezole, tolazoline, and yohimbine are commercially available α_2-antagonists that act both centrally and peripherally. They thus have potential to reverse the sedation as well as the peripheral effects induced by α_2-agonists. Atipamezole has a relatively high α_2:α_1 affinity ratio

(8300:1) compared to idazoxan (27:1), another α_2-antagonist in research use, and to yohimbine (40:1) (see review in Pertovaara et al. 2005).

In horses, atipamezole reverses the sedation, bradycardia, and atrioventricular blocks induced by detomidine (Nilsfors and Kvart 1986; Raekallio et al. 1990; Ramseyer et al. 1998; Knych and Stanley 2014) and medetomidine (Yamashita et al. 1996; Bettschart-Wolfensberger et al. 1999b). The reversal of bradycardia has, however, been shown to be transient, with the HR decreasing again after 5–10 min (Raekallio et al. 1990; Yamashita et al. 1996; Ramseyer et al. 1998). Also, re-sedation (Nilsfors and Kvart 1986; Ramseyer et al. 1998) or an incomplete reversal of sedation (Raekallio et al. 1990) have been observed with atipamezole administered after detomidine. Similarly, detomidine- and xylazine-induced sedation and bradycardia have been reversed with yohimbine and tolazoline (Kollias-Baker et al. 1993; Carroll et al. 1997; DiMaio Knych et al. 2012; Knych and Stanley 2014). Similarly to atipamezole, light re-sedation was observed following the initial reversal of detomidine sedation with yohimbine (DiMaio Knych et al. 2012). A decrease in SAP was observed when atipamezole was administered after detomidine (Nilsfors and Kvart 1986), while tolazoline after xylazine induced mild hypertension (Kollias-Baker et al. 1993).

Adverse effects, such as hyperexcitability and shivering, have been reported in horses receiving atipamezole after α_2-agonists (Ramseyer et al. 1998; Bettschart-Wolfensberger et al. 1999b). Moreover, Carroll et al. (1997) reported a stress response, sweating, and abnormally red mucous membranes after the reversal of detomidine-sedation with tolazoline. However, in another study, incremental doses of tolazoline reversed xylazine-induced sedation without adverse effects (Kollias-Baker et al. 1993).

Yohimbine alone has been demonstrated to increase the HR and induce variable behavioral changes; some of the horses appeared sedated, whereas most of them showed signs of agitation and even mild excitation (Kollias-Baker 1993; DiMaio Knych et al. 2012). Paradoxically, tolazoline alone reportedly decreases the HR and increases the number of atrioventricular blocks in horses (Casbeer and Knych 2013). The authors who reported this observation suggested that this effect could be related to increasing sympathetic tone and, consequently, a baroreflex-induced decrease in HR.

The use of these agents could be clinically advisable when, for example, the level of ataxia needs to be alleviated (Buchner et al. 1999) and in emergencies when the horse is collapsing or an α_2-agonist overdose needs to be treated (Di Concetto et al. 2007). It should, however, be noted that co-administered detomidine has been shown to decrease the volume of distribution and clearance of yohimbine (DiMaio Knych et al. 2012). Therefore, possible alterations in the pharmacokinetics of the drugs should be considered when they are administered together, as unexpected clinical responses may ensue (DiMaio Knych et al. 2012).

2.6.2 Vatinoxan

Vatinoxan (previously named MK-467 and L-659,066) is a hydrophilic α_2-receptor antagonist that mainly acts on peripheral receptors (Clineschmidt et al. 1988). The characteristics of it are interesting in animals because, unlike the centrally and peripherally acting antagonists, it has been shown to prevent certain peripheric effects of α_2-agonists (Pagel et al. 1998; Enouri et al. 2008), with minimal impact on their central effects, most importantly sedation, in dogs (Honkavaara et al. 2008; Restitutti et al. 2011; Rolfe et al. 2012), cats (Honkavaara et al. 2017a), sheep (Raekallio et al. 2010), and horses (Vainionpää et al. 2013; de Vries et al. 2016).

In rats and marmosets, vatinoxan crosses the BBB poorly, presumably due to its low lipid solubility (Clineschmidt et al. 1988). Its concentration in the brain has been shown to be 6%–7% of the concentration in plasma in rats after oral and i.v. administration and approximately 4% in marmosets after i.v. administration (Clineschmidt et al. 1988). Recently, Honkavaara et al. (in press) reported the concentration of vatinoxan to be 2% of that in plasma after i.v. administration in dogs. In other species, its concentration in the brain has not been definitely elucidated.

The α_2:α_1 receptor selectivity of vatinoxan in rats has been reported to be 105:1 and 29.5:1 *in vitro* and *in vivo*, respectively (Clineschmidt et al. 1988). The biotransformation or route of elimination of vatinoxan in different species has not been published. In an *in vitro* study, vatinoxan was moderately bound to canine plasma proteins and a racemic mixture of medetomidine did not influence this binding (Bennett et al. 2016). The elimination half-life of vatinoxan in dogs (250 μg/kg i.v.) has been reported to be 39–66 min (Honkavaara et al. 2012; Bennett et al. 2016) and in cats 122 min (300 μg/kg i.v.) (Pypendop et al. 2017). In horses, the elimination half-life was somewhat longer, 141 ± 29 min (200 μg/kg i.v.) (de Vries et al. 2016). Co-administered dexmedetomidine in dogs (Honkavaara et al. 2012) or romifidine in horses (de Vries et al. 2016) did not significantly influence the plasma concentration of vatinoxan.

2.6.2.1 Clinical effects of vatinoxan administered alone

Vatinoxan increases the HR when it is administered alone in humans (Schafers et al. 1992), rats (Szemeredi et al. 1989), dogs (Pagel et al. 1998; Enouri et al. 2008; Honkavaara et al. 2011), and horses (de Vries et al. 2016). In dogs, increased myocardial contractility has also been observed (Pagel et al. 1998). In rats and dogs, increased HR was accompanied with decreased MAP and SVR, respectively, and baroreflex activity along with increased sympathetic activity was thus suggested to be involved (Szemeredi et al. 1989; Pagel et al. 1998). de Vries et al. (2016) also suspected sympatho-adrenal activity as a reason for the increased HR because of the restlessness shown by some horses. Furthermore, RR was increased by vatinoxan as well (de Vries et al. 2016). Conversely, another study reported no influence on the HR or MAP by vatinoxan when administered alone in conscious horses (Bryant et al. 1998).

Vatinoxan alone (200 μg/kg i.v.) induced signs of abdominal discomfort (restlessness and kicking with the hind limbs towards the abdomen) and watery feces in some horses but was mostly well tolerated (de Vries et al. 2016). Mild to moderate abdominal discomfort has also been reported with certain doses of vatinoxan (> 140 μg/kg i.v.) in humans (Schafers et al. 1992). Furthermore, some dogs anesthetized with sevoflurane and receiving vatinoxan infusion during anesthesia exhibit post-anesthetic gastrointestinal side effects, such as vomiting and drooling (Hector et al. 2017).

2.6.2.2 Cardiopulmonary effects of vatinoxan with α_2-agonists

In dogs, premedication with vatinoxan has been reported to prevent the early cardiovascular effects of dexmedetomidine, such as increased SVR and MAP and decreased $\dot{Q}t$ (Pagel et al. 1998; Enouri et al. 2008). The later hypotensive effect of dexmedetomidine was preserved (Pagel et al. 1998). Similarly, in sheep, premedication with vatinoxan has been shown to attenuate the early cardiovascular changes induced by medetomidine (Bryant et al. 1998). In later studies, vatinoxan was administered concomitantly with dexmedetomidine to dogs (Honkavaara et al. 2011), sheep (Raekallio et al. 2010), and cats (Honkavaara et al. 2017a; 2017b), and a similar attenuating impact on hemodynamics was observed. Furthermore, in dogs dexmedetomidine-induced reduction in $\dot{D}O_2$ was improved by vatinoxan albeit the arterial blood gases were unaltered (Honkavaara et al. 2011). The effect of vatinoxan in dogs is dose-dependent (Pagel et al. 1998; Honkavaara et al. 2011), and a dexmedetomidine:vatinoxan ratio of 1:50 has been suggested to be optimal for i.v. administration in healthy dogs (Honkavaara et al. 2011).

Only a few studies have evaluated the effects of vatinoxan in combination with α_2-agonists in standing horses (Bryant et al. 1998; Vainionpää et al. 2013; de Vries et al. 2016; Pakkanen et al. 2018). In the study by Bryant et al. (1998), vatinoxan (264 μg/kg i.v.) administered before medetomidine (5 μg/kg i.v.) alleviated the bradycardia and partially also the hypertension caused by medetomidine. Similarly to dogs (Pagel et al. 1998), the hypotensive effect of medetomidine, as it is most probably centrally mediated, was preserved (Bryant et al. 1998). In the study by Vainionpää et al. (2013), the co-administration of vatinoxan (250 μg/kg i.v.) with detomidine (10 μg/kg i.v.) prevented the bradycardia and atrioventricular blocks and attenuated the increase in CVP that were observed with detomidine alone in standing horses. A slightly lower dose (200 μg/kg i.v.) of vatinoxan administered with romifidine (80 μg/kg i.v.) was found by de Vries et al. (2016) to alleviate the romifidine-induced atrioventricular blocks, the increase in the ABP, and the decrease in PvO_2. Heart rate tended to be higher in the presence of vatinoxan compared to romifidine alone.

2.6.2.3 Vatinoxan during general anesthesia

In dogs, vatinoxan administered as premedication with medetomidine before general anesthesia has been demonstrated to alleviate the medetomidine-induced early hypertension

and improve the decreases in CI and HR in a dose-dependent manner (Salla et al. 2017). The doses used in that study (medetomidine 10 μg/kg i.v. and vatinoxan 50–150 μg/kg i.v.) resulted in a clinically acceptable MAP during the anesthesia. In sheep, vatinoxan (150 μg/kg i.v.) prior to the administration of medetomidine (3 μg/kg i.v.) during sevoflurane-anesthesia has been shown to reduce SVR and MAP, but the sheep did not become hypotensive (Adam et al. 2018a).

In contrast to dogs (Salla et al. 2017) and sheep (Adam et al. 2018a), hypotension has been a concern in horses when vatinoxan has been administered as an adjunct to general anesthesia (Pakkanen et al. 2015; Wittenberg-Voges et al. 2018). In one study, vatinoxan (200 μg/kg i.v.) administered as premedication together with detomidine (20 μg/kg i.v.) before isoflurane anesthesia induced a higher HR, but MAP was significantly lower (Pakkanen et al. 2015). Wittenberg-Voges et al. (2018) administered vatinoxan (250 μg/kg i.v.) to horses during anesthesia that was maintained with isoflurane and a CRI of either xylazine (0.5 mg/kg loading dose and CRI rate of 1 mg/kg/h i.v.) or dexmedetomidine (3.5 μg/kg loading dose and CRI rate of 7 μg/kg/h i.v.). Similarly, vatinoxan-induced hypotension was observed, but CI was increased. The increased cardiac performance did not, however, sufficiently compensate for the hypotension, and the gastrointestinal microperfusion decreased with vatinoxan. Both of these studies concluded that the optimal agonist-antagonist dose ratio remains to be investigated in anesthetized horses.

2.6.2.4 Gastrointestinal effects of vatinoxan with α_2-agonists in horses

In a study by Vainionpää et al. (2013), gastrointestinal borborygmi, assessed by auscultation, were improved when vatinoxan was combined with detomidine in standing horses. Moreover, the horses passed feces during a 2-hour observation period with vatinoxan but not with detomidine alone. Similarly, in another study, decreases in the borborygmi score after romifidine were alleviated when vatinoxan was combined with it, and the horses passed feces more often during the experiment in the presence of vatinoxan (de Vries et al. 2016). On the other hand, in one canine study, the authors suggested that α_2-adrenoceptor antagonists, by preventing the sympathetic inhibition, could induce more frequent defecation as a result of increased migrating contractions of the colon (Maugeri et al. 1994).

2.6.2.5 Impact of vatinoxan on sedation

The impact of vatinoxan on sedation induced by α_2-agonists varies across studies but is generally considered minor. The sedation score of dogs was not found to be different between medetomidine alone or with concurrently administered vatinoxan i.v. and i.m. in a study by Rolfe et al. (2012). Conversely, in another study, vatinoxan administered simultaneously with medetomidine i.v. attenuated the antinociceptive effect of medetomidine and shortened the duration of sedation in dogs (Bennett et al. 2016). The discrepancy in sedation scores among studies has been attributed to different doses of drugs and differences in study protocols

(Bennett et al. 2016). In horses, concomitantly administered vatinoxan (200 μg/kg i.v.) and romifidine (80 μg/kg i.v.) have produced slightly lower median sedation scores than those achieved with romifidine alone, but the difference was not significant (de Vries et al. 2016). Furthermore, the area under the sedation score time curve was significantly smaller in horses when vatinoxan (250 μg/kg i.v.) was combined with detomidine (10 μg/kg i.v.) than with detomidine alone, even though the sedation scores at specific time points were not different between the treatments (Vainionpää et al. 2013).

2.6.2.6 Impact of vatinoxan on the pharmacokinetics of co-administered α_2-agonists

Pharmacokinetic studies have provided insight into the observed differences in sedation scores with and without vatinoxan. As vatinoxan is not supposed to reach the CNS in significant amounts (Clineschmidt et al. 1988, Honkavaara et al. in press), the observed effects on sedation and antinociception have been attributed to an altered disposition of concomitantly administered dexmedetomidine (Honkavaara et al. 2012; Bennett et al. 2016). Vatinoxan (i.v.) increased the apparent volume of distribution and clearance of i.v.-administered α_2-agonists—dexmedetomidine in dogs (Honkavaara et al. 2012) and detomidine (Vainionpää et al. 2013) and romifidine (de Vries et al. 2016) in horses. The blood flow of certain organs is reduced by dexmedetomidine in dogs (Restitutti et al. 2013). As vatinoxan has been shown to improve the blood flow (Restitutti et al. 2013), the increased volume of distribution and enhanced clearance of α_2-agonists (administered i.v.) with it have been attributed to the improved perfusion (Vainionpää et al. 2013; Bennett et al. 2016; de Vries et al. 2016). Furthermore, i.m.-administered vatinoxan enhanced the absorption of both medetomidine (Restitutti et al. 2017) and other drugs injected in the same syringe in dogs (Kallio-Kujala et al. 2018) and sheep (Adam et al. 2018b).

2.7 Anticholinergic drugs in treating bradycardia in horses

2.7.1 Cholinergic receptors

Cholinergic receptors are part of the autonomic nervous system, and their natural ligand is acetylcholine—the main neurotransmitter in the parasympathetic system and the preganglionic nerve ends of the sympathetic system (see review in Wehrwein et al. 2016). They can be divided into nicotinic and muscarinic receptors (Rang et al. 2016b). Muscarinic receptors mainly transmit the effects of acetylcholine at the postganglionic parasympathetic nerve endings. Additionally, muscarinic receptors indirectly mediate vasodilation (via inducing the production of nitric oxide) and sweating via cholinergic fibers of the sympathetic nervous system in the sweat glands (Rang et al. 2016b).

Muscarinic receptors are GCPR and have been divided into five subtypes, namely M_1–M_5 (review in Caulfield 1993). All of these receptor subtypes are variably expressed in the

cardiovascular system (see review in Saternos et al. 2018). Earlier studies suggested that the predominant receptor subtype in the heart was M_2, but later research has revealed that other subtypes are expressed as well (see reviews in Levey 1993, Saternos et al. 2018). Furthermore, differences in receptor expression may occur across species (Saternos et al. 2018). In the vasculature, muscarinic receptors mediate both vasoconstriction and vasodilation directly and indirectly (Saternos et al. 2018). Subtypes M_2 and M_3 have been reported to be mainly responsible for smooth muscle contraction in the intestines of horses (Marti et al. 2005), mice, and dogs (see review in Tobin et al. 2009).

2.7.2. Anticholinergic drugs in horses

Anticholinergic drugs, with antimuscarinic drugs (muscarinic antagonists) in particular, are drugs that inhibit cholinergic receptors and are typically used in horses to treat bradycardia during anesthesia (Schauvliege and Gasthuys 2013). Another clinical application in equine practice is relaxing the gastrointestinal smooth muscle to enable easier rectal palpation in horses with colic (Roelvink et al. 1991). The most commonly used antimuscarinic drugs for this purpose in horses are atropine, hyoscine-N-butylbromide (also called as hyoscine or scopolamine), and glycopyrrolate (Schauvliege and Gasthuys 2013). Atropine and hyoscine are naturally occurring muscarinic antagonists. Hyoscine is used as a molecule containing ammonium-group, hyoscine butylbromide, that does not cross the BBB.

2.7.2.1 Cardiovascular effects

Overall, vagally-mediated bradycardia related to α_2-agonists can be successfully treated with different anticholinergic drugs in standing (Alitalo et al. 1986; Gasthuys et al 1990; Pimenta et al. 2011; Perotta et al. 2014) and anesthetized horses (Teixeira Neto et al. 2004). Heart rate is increased by a direct blockade of the parasympathetic control of the heart and by a blockade of the vagally-mediated baroreflex (Hamlin et al. 1972). Stroke volume often decreases (Gasthuys et al. 1990; Geimer et al. 1995; Pimenta et al. 2011), but due to increased HR, the $\dot{Q}t$ may remain unaltered (Pimenta et al. 2011; Perotta et al. 2014) or even increase (Teixeira Neto et al. 2004; Perotta et al. 2014). Hypertension is one concern in the use of anticholinergics, particularly in conjunction with α_2-agonists (Marques et al. 1998; Pimenta et al. 2011; Perotta et al. 2014). Atropine or hyoscine alone may not consistently influence the ABP (Gasthuys et al. 1990; Geimer et al. 1995).

Atropine (5–10 µg/kg i.v.) has been shown to prevent the bradycardia and 2nd degree atrioventricular block in standing horses treated with detomidine (Alitalo et al. 1986; Gasthuys et al. 1990) or xylazine (Gasthuys et al. 1990). Interestingly, atropine also induced some atrioventricular blocks in conscious horses before the α_2-agonist was administered (Gasthuys et al. 1990). Premedication with hyoscine (0.14 mg/kg i.v.) before medetomidine (Perotta et al. 2014) or romifidine (Marques et al. 1998) has also been demonstrated to increase the HR and induce hypertension in standing horses. With an i.m. administration (0.3 mg/kg i.m.) of

hyoscine, the magnitude of the changes was smaller but the duration longer when compared to an i.v. bolus (Perotta et al. 2014).

Pimenta et al. (2011) compared hyoscine and atropine in detomidine-treated horses and detected a moderate increase in HR with hyoscine (0.2 mg/kg i.v.), while this increase was larger and more prolonged after atropine (0.02 mg/kg i.v.). With both drugs, severe hypertension was coincidentally observed. Despite the hemodynamic changes in hyoscine- or atropine-treated horses receiving medetomidine (Perotta et al. 2014) or detomidine (Pimenta et al. 2011), no changes in blood gas analyses were detected.

Similarly to other anticholinergic drugs, pretreatment with glycopyrrolate (2.5 μg/kg i.v.) alleviates the bradycardia after xylazine in horses, but the ABP increases (Singh et al. 1997). During general anesthesia maintained with halothane and a CRI of xylazine, repeated doses of glycopyrrolate increased the MAP, HR, and $\dot{Q}t$, and these changes resulted in an improved $\dot{D}O_2$ (Teixeira Neto et al. 2004).

2.7.2.2. Gastrointestinal effects in horses

Gastrointestinal hypomotility and consequent colic may limit the use of anticholinergics in horses (Ducharme and Fubini 1983; Teixeira Neto et al. 2004; Pimenta et al. 2011). Atropine has been experimentally shown to decrease intestinal motility for up to 12 h, and in clinical cases of colic, its use resulted in worsened gastrointestinal ileus and a delayed decision of surgical treatment in some individual horses (Ducharme and Fubini 1983). Hyoscine, in turn, reduced gastrointestinal motility for up to 60 min in standing horses (Sundra et al. 2012).

In the study by Pimenta et al. (2011) with detomidine-treated horses, atropine, but not hyoscine, further decreased the gastrointestinal auscultation scores. Furthermore, two horses receiving atropine developed abdominal distention. The total fecal transit time, however, was not altered (Pimenta et al. 2011). Glycopyrrolate has also been shown to decrease the gastrointestinal motility and to delay the return to normal borborygmi after xylazine (Singh et al. 1997). Furthermore, the administration of glycopyrrolate during general anesthesia has been reported to delay the recovery of normal borborygmi after anesthesia, and one of the horses receiving glycopyrrolate developed clinical signs of colic (Teixeira Neto et al. 2004).

3 AIMS OF THE STUDY

1. To evaluate the effects of vatinoxan on cardiopulmonary function during a CRI of medetomidine in standing horses (study I)

2. To investigate the effects of vatinoxan on cardiopulmonary function in horses anesthetized with isoflurane and a CRI of medetomidine (study II)

3. To compare the effects of vatinoxan and hyoscine on the cardiopulmonary changes induced by detomidine in horses when vatinoxan and hyoscine are administered after detomidine (study III)

4. To evaluate the effects of vatinoxan on the intestinal hypomotility associated with detomidine, a CRI of medetomidine, and general anesthesia in horses (studies I–III)

5. To determine the impact of vatinoxan on sedation induced by a medetomidine CRI and detomidine as well as on the concentrations of concurrently administered drugs in plasma (studies I–III)

4 MATERIALS AND METHODS

4.1 Study design

The design of all three studies (I–III) was an experimental, randomized cross-over study design. Randomization was performed by blindly assigning the treatment order to each horse, starting alternately with each treatment (studies I and II) and by a Latin square design (study III). The investigators assessing the sedation level and gastrointestinal borborygmi (studies I–III) and administering the dobutamine infusion (study II) were blinded to the assigned treatment.

4.2 Horses and baseline measurements

The study was approved by the National Animal Experiment Board of Finland (no. ESAVI/4789/04.10.07/2014).

Six adult, faculty-owned horses were used for the all 3 studies. The study horses included 3 mares and 3 geldings, representing Warmbloods (2 horses) and Standardbreds (4 horses). The median age was 10 years (range 5−19) and the median body weight 517 kg (range 451−614). The horses were considered healthy based on a physical examination as well as routine hematology and serum biochemistry. Prior to the start of the study, the right common carotid arteries were surgically translocated subcutaneously (Tapio et al. 2017). The operation was performed standing with sedation and local anesthesia, and the horses were allowed to recover from the procedure for a minimum 2 weeks before the beginning of the experiments. Between the experiments, the horses were housed in individual stalls with a daily turnout to a small paddock, and they were fed hay (8 kg a day) and had free access to water.

Prior to the start of the study, the baseline of fecal output was determined for each horse. During 3 consecutive days, each pile of feces was collected into a separate plastic bag and weighed. The daily fecal output (kg) and the daily number of fecal piles were then determined. The baseline for the monitored cardiopulmonary parameters was obtained for each horse on the day of each experiment, with the exception of study II. For study II, due to practical reasons, the median of 3 separate measurements for each horse on different days was used as a single baseline for the given parameter (these data were obtained from the measurements performed for study III).

4.3 Instrumentation

Instrumentation was achieved with the horses standing restrained in stocks prior to the administration of any drugs on each experiment day and removed at the end of each day.

Catheters were aseptically placed with subcutaneously infiltrated local anesthesia. An i.v. catheter (Intraflon 12G, Laboratoires Pharmaceutiques Vygon) was placed into the left jugular vein for drug administration. A central venous catheter (Cavafix Certo 12G, B. Braun) was placed more distally via the same jugular vein into the cranial vena cava. The pulmonary artery was catheterized (Arrow-Berman angiographic catheter 13G, Arrow International) through a large-bore i.v. catheter (Mila 10G, Mia International) in the right jugular vein (studies II and III). Characteristic pressure wave forms were observed on the screen (S/5 compact Critical Care Monitor, Datex Ohmeda) to confirm the correct location of the central venous and pulmonary arterial catheters. A mercury manometer was used to verify transducer accuracy. An arterial catheter (BD arterial cannula 20G, Becton-Dickinson Critical Care Systems) was introduced into the right common carotid artery. In study II the arterial catheter was placed after the induction of the anesthesia, in order to avoid the dislodgment of the catheter and the formation of a hematoma during the induction.

An electrocardiogram was connected to the horses and monitored in real time (S/5 compact Critical Care Monitor, Datex Ohmeda), but not recorded.

Cardiac output was determined by the lithium dilution method (LiDCO Plus, LidCO) as previously described (Hallowell and Corley 2005). A lithium dilution sensor was connected to the arterial catheter, and 1.875 mL of 8M lithium chloride was added to 100 mL of saline (0.9% NaCl) solution to obtain a lithium concentration of 150 mmol/L. During the measurements, a hemoglobin value of 10 g/dL and sodium value of 140 mmol/L were used. These values were corrected after the measurements with the formula provided by the manufacturer to match the true values measured from each horse at each time point.

4.4 Treatments and doses

In study I standing horses were restrained in stocks during the experiment and received 2 treatments at least a week apart. Medetomidine hydrochloride (Dorbene 1 mg/mL, Vetcare) 7 µg/kg was administered i.v. alone (MED) or with vatinoxan hydrochloride (MED+V) 140 µg/kg. The total injection volume was 10 mL, with the drugs diluted in 0.9% NaCl. Five min later, a medetomidine CRI was initiated at a rate of 3.5 µg/kg/h and continued for 60 min via a syringe infusion pump (Perfusor, B. Braun).

In study II the horses were premedicated for general anesthesia with medetomidine hydrochloride (Dorbene 1 mg/mL, Vetcare) 7 µg/kg i.v. alone (MED_{GA}) or with vatinoxan hydrochloride 140 µg/kg ($MED+V_{GA}$), with a minimum of 2 weeks between the treatments. The medications were diluted in 0.9% NaCl ad 20 mL. The horses were walked into an

induction room, and general anesthesia was induced 10 min later, at T0, with midazolam (Midazolam Accord 5 mg/mL; Accord Healthcare Ltd.) 0.06 mg/kg and ketamine (Ketador 100mg/mL; Richter Pharma AG) 2.2 mg/kg administered i.v. over 15 s. The tracheas were intubated, the horses transferred onto a padded surgical table and placed in dorsal recumbency. A rebreathing circuit (Tafonius, Hallowell EMC) was then connected and the administration of isoflurane in 100% oxygen initiated. The expiratory isoflurane was set to 1.2 vol% and adjusted in the presence of spontaneous nystagmus or an absence of the palpebral reflex by 0.1 vol%. The tidal volume was set to 12 mL/kg, and mechanical ventilation initiated at rate of 8 breaths/min. Ventilation was adjusted as necessary to maintain a target P_ECO_2 level of 35−55 mmHg. An adjunctive CRI of medetomidine at a rate of 3.5 µg/kg/h was started 10 min after the induction (at T10) and continued for 60 min (until T70). A dobutamine CRI (Dobuject 50 mg/mL, Primex Pharmaceuticals) was administered with a syringe infusion pump (Perfusor, B. Braun) according to a predefined plan to maintain the MAP within the target range of 70−80 mmHg. The dobutamine CRI was initiated at a rate defined by the first MAP reading (Table 1), and the rate was then doubled or halved every 5 min until the target range was achieved. If the MAP was more than 90 mmHg, the dobutamine CRI was discontinued completely. Ringer´s solution (Fresenius Kabi AB) was infused with an infusion pump (Colleague Volumetric Infusion Pump, Baxter) at 3 mL/kg/h i.v. throughout the anesthesia. At T70, immediately after the discontinuation of the medetomidine CRI, isoflurane was diminished for 10 min with the horses on the table. The horses were then transferred to the recovery box, the intubation tube was removed in the presence of the swallowing reflex, and the horses were allowed to recover unassisted.

Table 1. *Starting rate of dobutamine infusion defined by the initial mean arterial pressure value obtained from the arterial catheter installed in the right common carotid artery.*

Mean arterial pressure (mmHg)	Rate of dobutamine infusion (µg/kg/min)
60–69	0.5
50–59	1
40–49	2
< 40	4

Study III was conducted with the standing horses restrained in stocks. Three treatments were administered, with a minimum of 1-week wash-out period. We first administered detomidine hydrochloride (Equisedan, Vetcare) 20 µg/kg i.v. for all horses and, 10 min later, either vatinoxan 150 µg/kg (DET+V), hyoscine butylbromide (Buscopan, Boehringer-Ingelheim Intl) 0.2 mg/kg (DET+H), or saline (0.9% NaCl) (DET) i.v. Hyoscine served as a positive control (Pimenta et al. 2011) and NaCl as a negative control.

4.5 Data collection

4.5.1 Assessment of cardiopulmonary function

The cardiopulmonary data (Table 2) was collected for 120, 70, and 90 min in studies I, II, and III, respectively. Heart rates were determined by auscultation (studies I and III) or recorded from the electrocardiogram (Tafonius, Hallowell EMC) (study II). The RR was determined by observing the excursion of the chest wall (studies I and III).

In all studies, arterial and venous blood samples were drawn at the same points with $\dot{Q}t$ measurements and stored in ice water for no longer than 10 min until analysis for blood gases. In study I the venous blood sample was obtained from the central venous catheter, and in studies II and III, mixed venous blood drawn from the pulmonary arterial catheter was used for the analysis.

The cardiopulmonary calculations were performed using standard equations (Boyd et al. 1991; Hardman and Aitkenhead 2003; Haskins et al. 2005; Nyman et al. 2009; Menzies et al. 2016). Hemoglobin oxygen saturation was calculated using the oxygen dissociation curve of a horse (Clerbaux et al. 1993).

4.5.2 Assessment of gastrointestinal function

The motility of the gastrointestinal tract of the standing horses was assessed by auscultation of the borborygmi in studies I and III (Table 2). The borborygmi were scored based on a previously defined scoring system (Mama et al. 2009). Briefly, each of the 4 quadrants of the abdomen were auscultated for 30 s, and the number of rumbles was counted. The total number of rumbles was then used as an overall score. Additionally, in study III and as a sole gastrointestinal assessment in study II, the total fecal output was measured during the following 24 h after the experiment. Feces were collected and weighed in a similar manner as described for the baseline measurement. The time from the recovery from the anesthesia (study II) and from the end of the experiment (study III) to the first pile of feces was recorded.

Table 2. *Assessment of the cardiopulmonary and gastrointestinal function of the horses.*

Cardiopulmonary monitoring			**Calculated cardiopulmonary parameters**			**Gastrointestinal monitoring**		
Study I	**Study II**	**Study III**	**Study I**	**Study II**	**Study III**	**Study I**	**Study II**	**Study III**
HR	HR	HR	CI	CI		Auscultation score	Fecal output	Auscultation score
RR	RR	RR	SVI	SVI	SV			Fecal output
Carotid ABP	Carotid ABP	Carotid ABP	SVRI	SVRI	SVR			
CVP	CVP	CVP	LVW	LVW	LVW			
$\dot{Q}t$	$\dot{Q}t$	$\dot{Q}t$	$\dot{D}O_2I$	$\dot{D}O_2I$	$\dot{D}O_2$			
	PAP	PAP	$\dot{V}O_2I$	$\dot{V}O_2I$	$\dot{V}O_2$			
Arterial and venous blood gas analysis	Arterial and mixed venous blood gas analysis	Arterial and mixed venous blood gas analysis	O_2ER	O_2ER	O_2ER			
				PVRI	PVR			
	$P_{\bar{E}}CO_2$			Venous admixture				
	ET_{iso}			VD_{alv}:VT				
	Amount of dobutamine							

4.5.3 Assessment of the level of sedation

The sedation was scored using a previously defined scoring system in all 3 studies (Rohrbach et al. 2009). Briefly, a numerical rating scale was given for the attitude (0−3), standing ability (0−3), and eye lid aperture of the horse (0−2) and the position of its head (0−1) and ears (0−1) and these numerical values were summed to obtain a total sedation score (0−10, 10 representing the maximum level of sedation).

4.5.4 Assessment of the quality of induction and recovery (study II)

The induction time was recorded, as were the times of different stages of the recovery from the anesthesia. The induction was scored on a numerical rating scale from 0 (good; smooth induction) to 3 (poor; redosing is necessary to achieve recumbency) using a previously defined scoring system (Rossetti et al. 2008).

4.5.5 Drug concentrations in plasma

Plasma drug concentrations were mainly determined from arterial blood. As an exception, in study II at 1 min before the induction, mixed venous samples were used, because the horses did not have arterial catheters at that point. The first 5−10 mL of the blood samples were discarded and blood then drawn into EDTA-containing tubes (10 mL) at specific time points (Table 3). The plasma was separated by centrifuging the samples within 6 h of the sample collection and stored at -20°C until analysis. The concentrations of all administered drugs, except for dobutamine, in each experiment were analyzed. High-performance liquid chromatography-tandem mass spectrometry was used for the analysis, and the samples were processed in separate aliquots using specific conditions for each drug.

Table 3. *Drugs analyzed for plasma concentration.*

Study	**I**	**II**	**III**
Time points (min)	10, 30, 60, 90, 120	-1, 20, 40, 70	15, 30, 45, 60, 90
Analyzed drugs	Dexmedetomidine Levomedetomidine Vatinoxan	Dexmedetomidine Levomedetomidine Vatinoxan Midazolam Ketamine	Detomidine Vatinoxan Hyoscine

4.6 Statistical analysis

Statistical analyses were performed using commercially available software (GraphPad Prism, GraphPad Software Inc; SAS, SAS Institute Inc; SPSS, IBM), partially with the assistance of professional statisticians (studies I and II). For all studies, a *p*-value of <0.05 was considered statistically significant.

The normality assumptions of the drug concentration in plasma (studies I–III), the amount of dobutamine (study II), induction and recovery times (study II), total fecal output (studies II–III), and all parameters of study III were checked with Shapiro-Wilk tests. Kolmogorov-Smirnov tests were used to verify the normality assumptions of all other parameters of studies I and II.

In studies I and II, a change from baseline was used as the response with most cardiopulmonary variables—excluding those measured only during anesthesia. The differences between treatments in the change from baseline values were assessed with repeated measures analysis of covariance models. The model consisted of a baseline covariate, the main effects of treatment, period, sequence, time point of measurement, as well as two-way interactions of period*time point, sequence*time point, and treatment*time point as fixed effects; and of the main effect of horse as well as two-way interactions of period*horse and time point*horse as random effects. Estimates of treatment effect and within-treatment changes were calculated from the fitted models over time and by time point using model contrasts. For the variables measured only during anesthesia (study II), the same model was used, excluding the baseline covariate and using the actual values instead of the change as the response. For PAP (study II), the normal distribution of data could not be confirmed and, thus, Wilcoxon signed-rank sum testing by time point was performed. The areas under sedation/borborygmi score time curve (study I) and plasma drug concentration time curve (studies I and II) were calculated. The differences between and within treatments for all scores in studies I and II were investigated with the Wilcoxon signed-rank sum test. For the plasma drug concentration (studies I and II), total amount of dobutamine, induction and recovery times, and fecal output variables (study II), the differences between treatments were analyzed with a paired t test.

In study III, for physiological variables measured over time, differences between the three treatments were assessed by two-way repeated measures analysis of variance with Tukey *post hoc* tests. Comparisons within each treatment were performed with Dunnett *post hoc* tests. The areas under the sedation/borborygmi score and plasma drug concentration time curves were calculated. The differences between and within treatments in the sedation/borborygmi scores and fecal output variables were investigated with the Friedman test. Repeated measures analysis of variance and paired *t* tests were used to investigate the differences between treatments in pharmacokinetic variables for detomidine.

5 RESULTS

5.1 Cardiopulmonary function in standing horses (studies I and III)

The cardiopulmonary results of study I are summarized in Table 4 and Figure 1. In study I, vatinoxan attenuated some of the cardiopulmonary changes induced by a loading dose and CRI of medetomidine, particularly in the beginning of the observation period. With MED, significant changes in SVRI and CI were observed, while no changes from baseline were detected with MED+V. There were no significant differences in SV between MED and MED+V, although SV increased significantly with MED after the discontinuation of the CRI. With MED+V, MAP immediately decreased from the baseline value, whereas a slow decrease in MAP was detected with MED. Of the oxygenation parameters (PaO_2, PvO_2, and $\dot{D}O_2I$), only PvO_2 differed significantly between the treatments, being lower (compared to baseline) with MED. Transiently, O_2ER increased significantly more from baseline with MED, but $\dot{V}O_2I$ differed between the treatments only at 120 min, increasing significantly more from baseline with MED than with MED+V.

The cardiopulmonary results of study III are presented in Figure 2 and in Tables 5 and 6. Overall, several variables differed significantly between the treatments after 15 min (i.e. after the administration of the treatment). The decrease in HR and $\dot{Q}t$ were reversed by both vatinoxan and hyoscine, but after hyoscine administration, the bradycardia turned into tachycardia and the LVW increased significantly. After the administration of vatinoxan, blood pressures decreased along with SVR, whereas hyoscine transiently exacerbated the increase in carotid and pulmonary ABP. Stroke volume decreased significantly with hyoscine, while no SV changes were detected with the other treatments. Additionally, PVR was highest after hyoscine administration compared to the other two treatments. The detomidine-induced decrease in $P\bar{v}O_2$ was delayed in the presence of vatinoxan, but the decrease in $\dot{D}O_2$ was unaffected by any of the treatments.

Table 4. Mean (± standard deviation) cardiopulmonary data after medetomidine hydrochloride (7 µg/kg i.v.) without (MED) or with (MED+V) vatinoxan (140 µg/kg i.v.), followed by a CRI of medetomidine hydrochloride (3.5 µg/kg/h) continued for 60 min (study I). Significant differences ($p<0.05$) are labelled at the given time points as * between treatments and § within treatment in relation to baseline.

Variable	Treatment	Time						
		Baseline	**10 min**	**20 min**	**30 min**	**60 min**	**90 min**	**120 min**
HR (beats/min)	MED	40 ± 11	26 ± 2§	27 ± 3§	27 ± 3§	28 ± 1§	32 ± 3§	32 ± 3§
	MED+V	36 ± 6	31 ± 5*§	31 ± 3*§	31 ± 3§	30 ± 3§	31 ± 3§	39 ± 14§
MAP (mmHg)	MED	128 ± 18	129 ± 15	124 ± 14	118 ± 14	115 ±14§	99 ± 10§	97 ± 10§
	MED+V	125 ± 15	103 ± 13*§	102 ± 15*§	100 ± 19*§	102 ± 13*§	105 ± 15§	115 ± 12*§
Mean CVP (mmHg)	MED	11 ± 4	14 ± 3§	12 ± 2	12 ± 4	10 ± 2	10 ± 4	8 ± 4§
	MED+V	12 ± 3	10 ± 3*	9 ± 4*	7 ± 4*§	8 ± 4§	9 ± 3§	11 ± 4
RR (breaths/min)	MED	20 ± 9	12 ± 5§	10 ± 2§	12 ± 5§	8 ± 2§	8 ± 1§	8 ± 3§
	MED+V	15 ± 4	8 ± 1§ *	9 ± 2§	7 ± 2§ *	7 ± 2§	6 ± 2§	7 ± 3§
PaO_2 (mmHg)	MED	100 ± 4	94 ± 6§	-	95 ± 4§	99 ± 4	102 ± 7	106 ± 5§
	MED+V	99 ± 9	92 ± 3§	-	98 ± 4	99 ± 4	104 ± 3§	100 ± 4*
PvO_2 (mmHg)	MED	36 ± 6	26 ± 2§	-	28 ± 2§	29 ± 2§	31 ± 3§	30 ± 3§
	MED+V	34 ± 4	33 ± 2§ *	-	32 ± 3§ *	31 ± 3§	31 ± 3§	36 ± 4*
$\dot{D}O_2I$ (mL/min/kg)	MED	15 ± 5	8 ± 1§	-	9 ± 1§	10 ± 2§	14 ± 6	20 ± 8§
	MED+V	14 ± 3	11 ± 1	-	10 ± 3§	10 ± 2§	10 ± 2§	16 ± 5
O_2ER (%)	MED	24 ± 8	41 ± 5§	-	36 ± 5§	35 ± 4§	31 ± 5§	35 ± 6§
	MED+V	28 ± 7	28 ± 4*	-	30 ± 6§ *	32 ± 6§	33 ± 6§	24 ± 7*

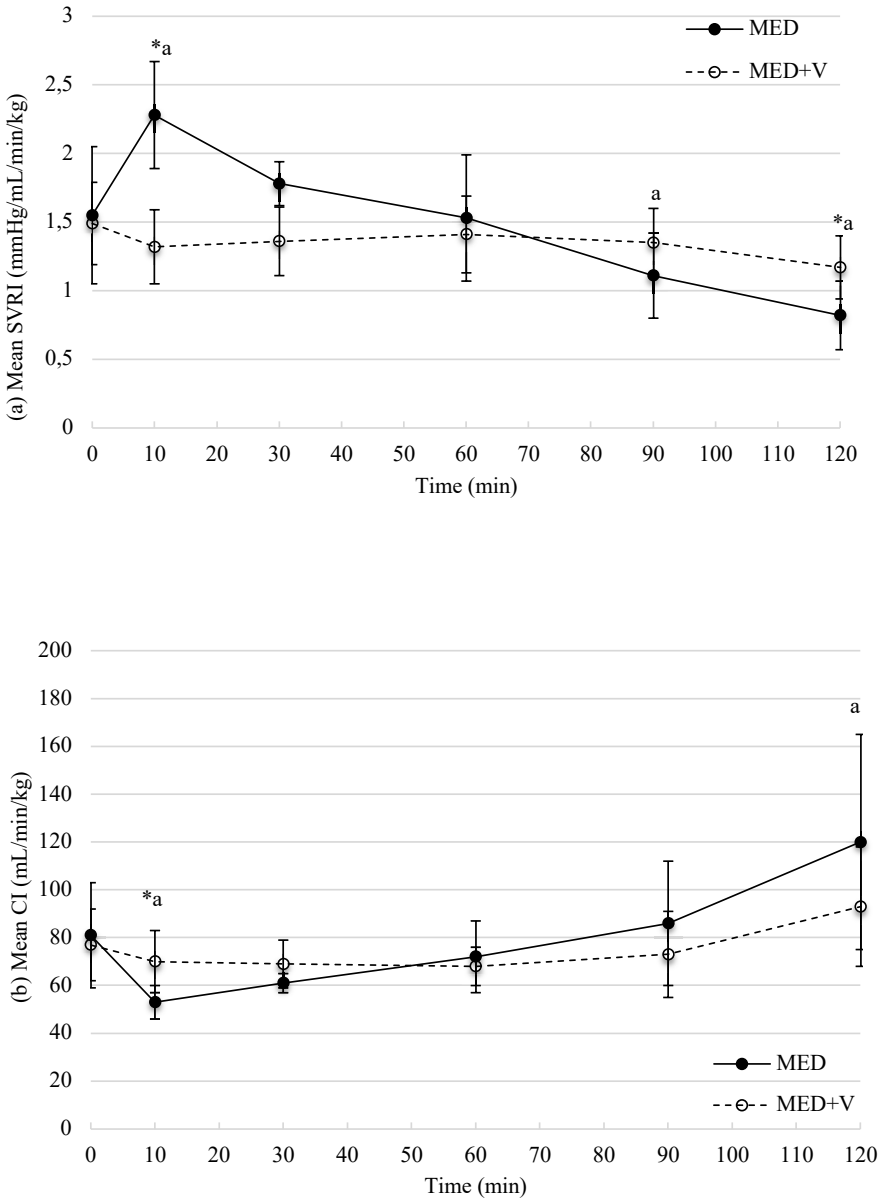

Figure 1. *Mean SVRI (a) and CI (b) after medetomidine hydrochloride administration (7 µg/kg i.v.) without (MED) or with (MED+V) vatinoxan (140 µg/kg i.v.), followed by a CRI of medetomidine hydrochloride (3.5 µg/kg/h) continued for 60 min (study I). * indicates significant difference (p<0.05) between treatments and (a) within MED with respect to baseline at a given time point. Error bars indicate standard deviation.*

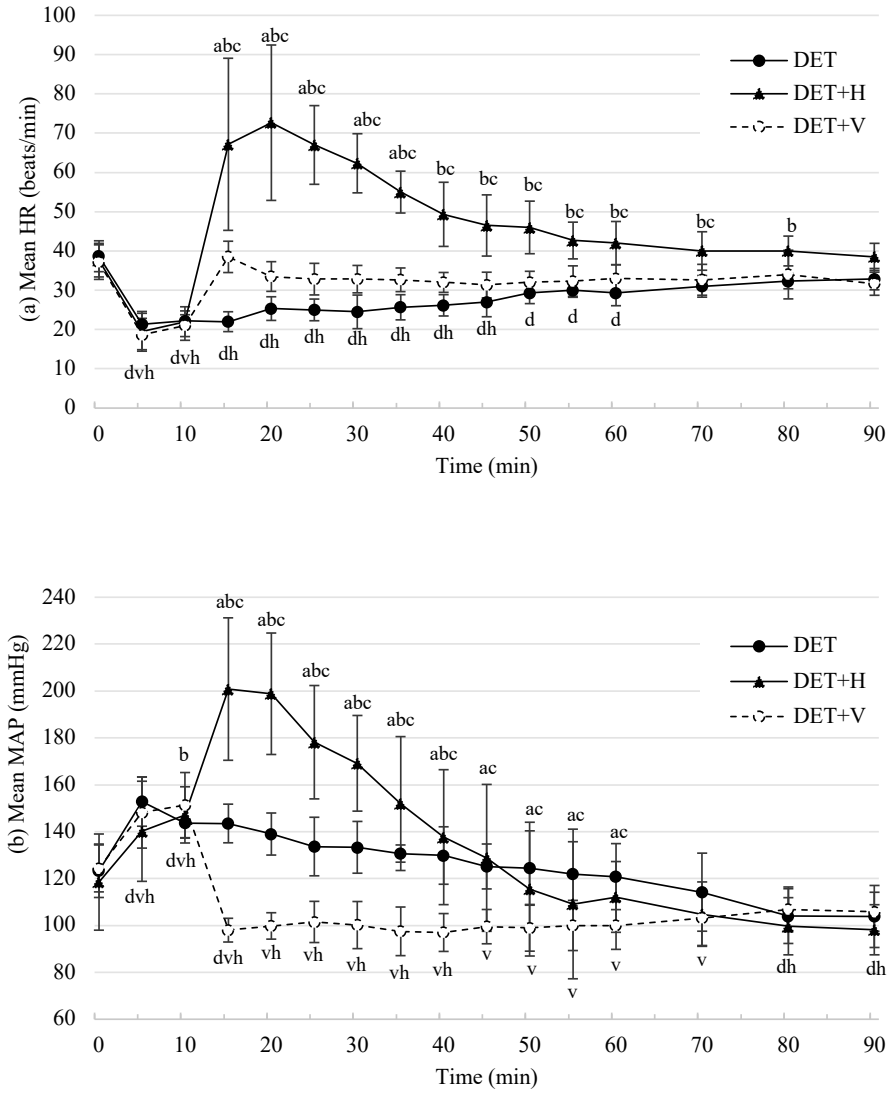

Figure 2. *Mean HR (a) and MAP (b) after detomidine administration (20μg/kg i.v.) at 0 min and the co-administration of saline (DET), hyoscine butylbromide (0.2 mg/kg i.v.) (DET+H), or vatinoxan (150 μg/kg i.v) (DET+V) at 10 min (study III). Error bars indicate standard deviation. Significant differences (p<0.05) between treatments at the given time points are labeled as (a) between DET and DET+V, (b) between DET and DET+H, and (c) between DET+V and DET+H; and significant within-treatment differences with respect to baseline as (d) within DET, (v) within DET+V, and (h) within DET+H.*

Table 5. *Mean (± standard deviation) cardiopulmonary parameters after detomidine administration (20μg/kg i.v.) at 0 min and the co-administration of saline (DET), vatinoxan (150μg/kg i.v) (DET+V), or hyoscine butylbromide (0.2 mg/kg i.v.) (DET+H) at 10 min (study III). Significant differences (p<0.05) are labelled at the given time points as [§] within treatment with respect to baseline. Values with different superscript letters [(a,b,c)] differ significantly (p<0.05) from each other at the given time point.*

Variable	Treatment	Time				
		Baseline	**15 min**	**30 min**	**60 min**	**90 min**
Mean CVP (mmHg)	DET	10 ± 4	14 ± 8^{a§}	17 ± 4^{a§}	9 ± 3^{a}	6 ± 5^{a,b}
	DET+V	8 ± 3	9 ± 5^{b}	8 ± 5^{b}	7 ± 3^{a}	8 ± 4^{b}
	DET+H	11 ± 4	11 ± 6^{b}	2 ± 5^{c§}	2 ± 4^{b§}	3 ± 2^{a§}
Mean PAP (mmHg)	DET	27 ± 5	30 ± 5^{a}	26 ± 6^{a}	23 ± 4^{a}	21 ± 5§
	DET+V	23 ± 3	24 ± 5^{b}	21 ± 2^{b}	19 ± 3^{b}	20 ± 3
	DET+H	24 ± 6	40 ± 7^{c§}	22 ± 4^{b}	17 ± 5^{b§}	17 ± 5§
Q̇t (L/min)	DET	42 ± 13	30 ± 12^{a}	31 ± 6	34 ± 5	37 ± 5
	DET+V	52 ± 13	50 ± 20^{b}	39 ± 10	38 ± 8	35 ± 3§
	DET+H	44 ± 26	42 ± 8^{b}	39 ± 7	34 ± 6	33 ± 7
SV (L/beat)	DET	1.1 ± 0.3	1.4 ± 0.6^{a}	1.3 ± 0.4^{a}	1.2 ± 0.2^{a}	1.1 ± 0.2
	DET+V	1.4 ± 0.3	1.3 ± 0.6^{a}	1.1 ± 0.1^{a}	1.2 ± 0.3^{a}	1.1 ± 0.1
	DET+H	1.1 ± 0.5	0.7 ± 0.3^{b§}	0.6 ± 0.2^{b§}	0.8 ± 0.2^{b}	0.9 ± 0.1
LVW (kg/m)	DET	70 ± 24	51 ± 17^{a}	57 ± 13^{a}	55 ± 11	52 ± 13
	DET+V	87 ± 25	66 ± 23^{a§}	53 ±11^{a§}	52 ± 12§	51 ± 8§
	DET+H	72 ± 50	112 ± 31^{b§}	91 ± 23^{b}	51 ± 13	44 ± 10§
SVR (dynes/s/cm^5)	DET	230 ± 60	356 ± 125^{a§}	304 ± 42^{a§}	270 ± 47^{a}	215 ± 29
	DET+V	190 ± 48	157 ± 46^{b}	197 ± 44^{b}	200 ± 34^{b}	222 ± 16
	DET+H	227 ± 82	370 ± 90^{a§}	345 ± 48^{a§}	268 ± 36^{a}	239 ± 55
PVR (dynes/s/cm^5)	DET	31 ± 18	20 ± 7^{a}	23 ± 12	26 ± 6	26 ± 9
	DET+V	26 ± 12	29 ± 9^{a}	32 ± 11	33 ± 8	33 ± 8
	DET+H	34 ± 19	47 ± 10^{b}	34 ± 12	36 ± 5	37 ± 18

Table 6. *Mean (± standard deviation) RR, arterial and mixed venous blood gas parameters, $\dot{D}O_2$, and $\dot{V}O_2$ after detomidine administration (20μg/kg i.v.) at 0 min and the co-administration of saline (DET), vatinoxan (150μg/kg i.v) (DET+V), or hyoscine butylbromide (0.2 mg/kg i.v.) (DET+H) at 10 min (study III). Significant differences ($p<0.05$) are labelled at the given time points as § within treatment with respect to baseline, and values with different superscript letters $^{(a,b,c)}$ differ significantly ($p<0.05$) from each other at the given time point.*

Variable	Treatment	Time				
		Baseline	**15 min**	**30 min**	**60 min**	**90 min**
RR (breaths/min)	DET	15 ± 6	14 ± 8^{a}	12 ± 4	7 ± 3§	7 ± 2§
	DET+V	14 ± 6	12 ± 3^{b}	10 ± 4	7 ± 1§	6 ± 1§
	DET+H	14 ± 4	12 ± 2^{b}	13 ± 5	8 ± 0§	6 ± 2§
PaO_2 (mmHg)	DET	107 ± 4	95 ± 12	98 ± 10	98 ± 6	105 ± 7
	DET+V	102 ± 9	94 ± 5	98 ± 4	102 ± 5	105 ± 7
	DET+H	106 ± 5	96 ± 9	92 ± 7§	91 ± 8§	99 ± 7
$P\bar{v}O_2$ (mmHg)	DET	39 ± 5	26 ± 3^{a§}	30 ± 2^{a§}	31 ± 2§	31 ± 3^{a,b§}
	DET+V	37 ± 3	40 ± 2^{b}	35 ± 1^{b}	33 ± 2§	33 ± 2^{a§}
	DET+H	36 ± 5	28 ± 3^{a§}	35 ± 4^{b}	31 ± 2§	29 ± 2^{b§}
$\dot{D}O_2$ (L/min)	DET	8.1 ± 2.9	5.2 ± 2.0§	5.1 ± 1.5	4.9 ± 0.6§	5.3 ± 1.0§
	DET+V	9.8 ± 2.9	8.7 ± 3.4	6.1 ± 1.6§	5.4 ± 1.0§	5.2 ± 0.6§
	DET+H	8.0 ± 5.1	7.1 ± 1.4	6.4 ± 1.3	4.6 ± 0.8§	4.5 ± 0.9§
$\dot{V}O_2$ (L/min)	DET	1.6 ± 0.4	2.2 ± 1.0	1.7 ± 0.5	1.5 ± 0.3	1.6 ± 0.3
	DET+V	2.0 ± 0.5	1.6 ± 0.5	1.5 ± 0.4	1.6 ± 0.5	1.5 ± 0.2
	DET+H	1.7 ± 0.5	2.8 ± 0.7§	1.6 ± 0.4	1.5 ± 0.2	1.6 ± 0.4

5.2 Cardiopulmonary function in anesthetized horses (study II)

The results regarding HR and carotid ABP are summarized in Table 7. Overall, significantly more dobutamine was infused to maintain MAP within the target range with MED+V_{GA} than with MED_{GA} (p = 0.018); the overall dobutamine consumption per horse was 27 ± 16 and 67 ± 25 µg/kg for MED_{GA} and MED+V_{GA}, respectively. The rate of dobutamine CRI varied between 0 and 1 µg/kg/min with MED_{GA} and between 0 and 4 µg/kg/min with MED+V_{GA}. The PAP differed between the treatments only at 30 and 40 min, at which point the systolic PAP was significantly higher with MED+V_{GA} than with MED_{GA}. The other cardiopulmonary parameters are provided in Table 8. The cardiac performance (i.e. CI, SVI, and LVW) decreased significantly more from baseline with MED_{GA} than with MED+V_{GA}. The SVRI increased significantly more from baseline with MED_{GA}, while no significant differences in PVRI were detected between or within treatments. Mixed venous oxygen tension and $\dot{D}O_2I$ decreased significantly more from baseline with MED_{GA} than with MED+V_{GA}. There were no differences in $\dot{V}O_2I$ between treatments, but in O_2ER the increase from baseline was significantly higher with MED_{GA}. No difference in parameters describing ventilation perfusion mismatch (venous admixture, VD_{alv}:VT) were detected between the treatments.

Table 7. *Mean (± standard deviation) HR and carotid ABP during general anesthesia maintained with a isoflurane and medetomidine CRI (3.5 μ g/kg/h) from 10 to 70 min. Horses received medetomidine hydrochloride (7 μ g/kg i.v.) alone (MED_{GA}) or with vatinoxan (140 μ g/kg i.v.) ($MED+V_{GA}$) 10 min before induction, which was administered at the time point of 0 min (study II). Significant differences ($p<0.05$) are labelled at the given time points as * between treatments and § within treatment in relation to baseline.*

Variable	Baseline	Treatment	Time						
			10 min	20 min	30 min	40 min	50 min	60 min	70 min
HR (beats/min)	37 ± 5	MED_{GA}	34 ± 4	29 ± 4§	31 ± 5	31 ± 4§	32 ± 5	30 ± 5§	31 ± 5
		$MED+V_{GA}$	42 ± 13*§	33 ± 5	35 ± 8	34 ± 4	35 ± 6	33 ± 5	36 ± 5
SAP (mmHg)	152 ± 12	MED_{GA}	102 ± 22§	102 ± 12§	95 ± 15§	98 ± 7§	95 ± 11§	106 ± 13§	92 ± 8§
		$MED+V_{GA}$	76 ± 16*§	112 ± 12§	123 ± 21*§	118 ± 27*§	111 ± 14§	120 ± 21§	111 ± 21*§
MAP (mmHg)	123 ± 11	MED_{GA}	77 ± 21§	80 ± 8§	68 ± 16§	74 ± 10§	71 ± 6§	82 ± 5§	75 ± 6§
		$MED+V_{GA}$	52 ± 12*§	83 ± 22§	76 ± 10§	80 ± 14§	71 ± 11§	79 ± 4§	73 ± 5§
DAP (mmHg)	101 ± 10	MED_{GA}	68 ± 24§	72 ± 10§	67 ± 10§	74 ± 7§	60 ± 7§	73 ± 6§	64 ± 5§
		$MED+V_{GA}$	41 ± 12*§	66 ± 23§	60 ± 8§	59 ± 9*§	58 ± 14§	62 ± 5§	62 ± 6§

Table 8. *Mean (± standard deviation) cardiopulmonary data during general anesthesia maintained with a isoflurane and medetomidine CRI (3.5 μg/kg/h) from 10 to 70 min. Horses received medetomidine hydrochloride (7 μg/kg i.v.) alone (MED_{GA}) or with vatinoxan (140 μg/kg i.v.) ($MED+V_{GA}$) 10 min before induction, which was administered at the time point of 0 min (study II). Significant differences ($p<0.05$) are labelled at the given time points as * between treatments and § within treatment in relation to baseline.*

Variable	Baseline	Treatment	Time		
			20 min	40 min	70 min
CI (mL/min/kg)	79 ± 19	MED_{GA}	39 ± 2§	47 ± 10§	46 ± 7§
		$MED+V_{GA}$	73 ± 18*	69 ± 15*	62 ± 26§
Mean CVP (mmHg)	10 ± 3	MED_{GA}	-9 ± 3§	-8 ± 4§	-8 ± 5§
		$MED+V_{GA}$	-4 ± 10§	-7 ± 5§	-6 ± 8§
LVW (kg•m/min)	71 ± 23	MED_{GA}	21 ± 2§	24 ± 7§	23 ± 2§
		$MED+V_{GA}$	42 ± 13*§	38 ± 10*§	31 ± 11§
SVI (mL/beat/kg)	2.1 ± 0.5	MED_{GA}	1.4 ± 0.2§	1.5 ± 0.3§	1.6 ± 0.3§
		$MED+V_{GA}$	2.4 ± 0.6	2.1 ± 0.3	1.8 ± 0.8
SVRI (mmHg/mL/min/kg (dynes/s/cm^5/kg))	1.4 ± 0.4 (118 ± 24)	MED_{GA}	2.2 ± 0.3§ (175 ± 27)	1.8 ± 0.3 (143 ± 26)	1.8 ± 0.4 (142 ± 29)
		$MED+V_{GA}$	1.3 ± 0.6 (103 ± 49)	1.3 ± 0.4 (105 ± 31)	1.6 ± 0.9 (126 ± 28)
PaO_2 (mmHg)	106 ± 3	MED_{GA}	287 ± 100§	278 ± 93§	250 ± 112§
		$MED+V_{GA}$	284 ± 109§	283 ± 142§	260 ± 118§
$P\bar{v}O_2$ (mmHg)	39 ± 3	MED_{GA}	33 ± 3	35 ± 4	35 ± 6
		$MED+V_{GA}$	42 ± 2*	47 ± 8*§	46 ± 10*§
$PaCO_2$ (mmHg)	40 ± 2	MED_{GA}	43 ± 3	45 ± 5	47 ± 5
		$MED+V_{GA}$	46 ± 4	49 ± 4	50 ± 6
$\dot{D}O_2I$ (mL/min/kg)	15 ± 4	MED_{GA}	7 ± 1§	9 ± 3§	8 ± 1§
		$MED+V_{GA}$	15 ± 5*	15 ± 4*	13 ± 7
O_2ER (%)	20 ± 3	MED_{GA}	33 ± 5§	29 ± 6§	28 ± 6§
		$MED+V_{GA}$	20 ± 2*	17 ± 6*	19 ± 8*
Hemoglobin (g/L)	136 ± 12	MED_{GA}	128 ± 12	133 ± 15	129 ± 13
		$MED+V_{GA}$	143 ± 14	153 ± 13§	147 ± 19

5.3 Gastrointestinal function (studies I–III)

The intestinal borborygmi score was higher in the presence of vatinoxan in studies I and III (Tables 9 and 10). The total 24-hour fecal output after detomidine sedation did not differ between any of the treatments (study III), nor was the decreased fecal output associated with general anesthesia affected by vatinoxan (study II). After general anesthesia (study II), the median (range) time to pass the first pile of feces was 270 (45–750) min and 270 (60–480) min with MED_{GA} and $MED+V_{GA}$, respectively, $p = 0.50$. In study III, the median time to the first feces after the treatment (at 10 min) was significantly shorter (130 min; range 60–200) for DET+V than for DET (220 min; range 160–350) $p = 0.015$.

Table 9. *Median (minimum, maximum) borborygmi score after medetomidine hydrochloride administration (7 µg/kg i.v.) without (MED) or with (MED+V) vatinoxan (140 µg/kg i.v.), followed by a CRI of medetomidine hydrochloride (3.5 µg/kg/h) continued for 60 min (study I). Significant differences (p<0.05) at the given time points are labeled as * between treatments and § within treatment in relation to baseline. AUC = area under borborygmi score time curve.*

Treatment	Time						$AUC_{0–120min}$
	Baseline	10 min	30 min	60 min	90 min	120 min	
MED	5.0 (3, 7)	1.3 (0, 2)§	0.3 (0, 1)§	0.0 (0, 1)§	0.5 (0, 1.5)§	1.5 (0.5, 2.5)§	93 (28, 168)
MED+V	4.5 (3, 8)	3.0 (2, 8)*	2.3 (1, 4)*§	2.5 (1, 3)*§	5.8 (2.5, 7)*	4.3 (2, 8)*	468 (236, 612)*

Table 10. *Median (minimum, maximum) borborygmi score after detomidine administration (20µg/kg i.v.) at 0 min and the co-administration of saline (DET), vatinoxan (150µg/kg i.v.) (DET+V), or hyoscine butylbromide (0.2 mg/kg i.v.) (DET+H) at 10 min (study III). Significant differences (p<0.05) at given time points are labeled as § within treatment in relation to baseline and the AUC values with different superscript letters [a,b] differ significantly (p<0.05) from each other at the given time point. AUC = area under the borborygmi score time curve.*

Treatment	Time						$AUC_{0–90min}$
	Baseline	5 min	15 min	30 min	60 min	90 min	
DET	5.0 (4, 7)	2.0 (0.5, 3.5)§	0.8 (0.0, 1.5)§	0.0 (0, 3)§	0.0 (0, 1)§	1.5 (1, 3)§	59 [a,b] (40, 185)
DET+V	5.0 (3.5, 7)	2.3 (1.5, 4.5)§	2.3 (2, 4)§	3.3 (1, 6.5)§	4.3 (4, 6)	4.0 (4, 5.5)	332[a] (281, 436)
DET+H	5.5 (2, 8)	2.0 (0.5, 3)§	0.3 (0, 1)§	0.0 (0, 0.5)§	0.3 (0, 1)§	1.0 (0.5, 1.5)§	59[b] (35, 74)

5.4 Quality of sedation and anesthesia (studies I–III)

In a standing horse, vatinoxan significantly reduced the sedation induced by a loading dose and a subsequent CRI of medetomidine for the first 10 min, after which no difference between treatments was detected anymore (study I, Table 11).

The median (minimum, maximum) sedation score 1 min before the induction of general anesthesia (study II) was 6.5 (6, 7) and 6.5 (5, 7) for MED_{GA} and $MED+V_{GA}$, respectively, $p = 0.414$. The ET_{iso} did not significantly differ between MED_{GA} and MED_{GA+V} (study II).

In study III, the sedation score returned to the baseline level sooner with DET+V than with DET or DET+H (Table 12).

Table 11. *Median (minimum, maximum) sedation score after medetomidine hydrochloride administration (7 µg/kg i.v.) without (MED) or with (MED+V) vatinoxan (140 µg/kg i.v.), followed by a CRI of medetomidine hydrochloride (3.5 µg/kg/h) continued for 60 min (study I). Significant differences (p<0.05) are labelled at the given time points as * between treatments and § within treatment in relation to baseline. AUC = area under sedation score time curve.*

Treatment	Base-line	Time						$AUC_{0–120\ min}$
		10 min	20 min	30 min	60 min	90 min	120 min	
MED	1 (0, 1)	7 (6, 8)§	7 (6, 7)§	6.5 (6, 7)§	5.5 (2, 7)§	2.5 (0, 6)	1.5 (0, 3)	513 (405, 695)
MED+V	0.5 (0, 1)	4.5 (3, 7)*§	5 (4, 7) §	5.5 (4, 7)§	4.5 (4, 6)§	2 (1, 4)§	1 (1, 2)§	428 (368, 515)

Table 12. *Median (minimum, maximum) sedation score after detomidine administration (20µg/kg i.v.) at 0 min and the co-administration of saline (DET), vatinoxan (150µg/kg i.v.) (DET+V), or hyoscine butylbromide (0.2 mg/kg i.v.) (DET+H) at 10 min (study III). Significant differences (p<0.05) are labelled at the given time points as § within treatments and the AUC values with different superscript letters [(a,b)] differ significantly (p<0.05) from each other at the given time point. AUC = area under sedation score time curve.*

Treatment	Base-line	Time						$AUC_{0–90\ min}$
		5 min	15 min	30 min	45 min	60 min	90 min	
DET	1 (0, 1)	7 (6, 7)§	7 (7, 8)§	7 (7, 7)§	6 (5, 7)§	3.5 (2, 7)§	2.5 (1, 3)§	449 (395,555)[a,b]
DET+V	1 (1, 1)	7 (6, 7)§	7 (6, 8)§	6 (4, 7)§	5 (5, 6)§	3.5 (2, 4)§	2 (1, 3)	428 (368, 463)[a]
DET+H	1 (0, 1)	7 (6, 8)§	7 (6, 8)§	7 (7, 8)§	6 (6, 7)§	4.5 (4, 7)§	2 (2, 4)§	486 (443, 563)[b]

5.5 Quality of induction and recovery (study II)

The median (range) induction score was 1 (1–1) and 2 (1–3) with MED_{GA} and $MED+V_{GA}$, respectively, without significant difference between the treatments ($p = 0.063$). The induction was, however, faster in the presence of vatinoxan—83 ± 11 and 63 ± 10 s ($p = 0.008$) with MED_{GA} and $MED+V_{GA}$, respectively. All recoveries were uneventful. The recorded recovery times did not differ between MED_{GA} and $MED+V_{GA}$.

5.6 Drug concentrations in plasma (studies I–III)

The drug concentrations in plasma and areas under the plasma concentration time curves (studies I and II) are presented in the Tables 13 and 14. The dexmedetomidine concentration in plasma and the areas under the plasma concentration time curves of dexmedetomidine and levomedetomidine were significantly lower with MED+V in study I (Table 13). In study II, the area under the plasma concentration time curve of ketamine, but not that of dexmedetomidine or levomedetomidine, was significantly decreased with $MED+V_{GA}$ compared to MED_{GA}.

The area under the plasma concentration time curve of detomidine was not significantly affected by the vatinoxan administered later (study III) (Table 15). The volume of the distribution of detomidine was significantly larger with DET+H than with to DET or DET+V.

Table 13. *Mean (± standard deviation) area under the plasma concentration time curve (AUC) of dexmedetomidine, levomedetomidine, and vatinoxan after medetomidine hydrochloride administration (7 µg/kg i.v.) without (MED) or with (MED+V) vatinoxan (140 µg/kg i.v.), followed by a CRI of medetomidine hydrochloride (3.5 µg/kg/h) continued for 60 min (study I).* * *Significant difference (p<0.05) between the treatments.*

Drug	Treatment	$AUC_{0–120\ min}$ (min · ng/mL)
Dexmedetomidine	MED	72.0 ± 18.0
	MED+V	58.3 ± 17.2*
Levomedetomidine	MED	29.3 ± 11.7
	MED+V	24.7 ± 11.7*
Vatinoxan	MED+V	8950 ± 2851

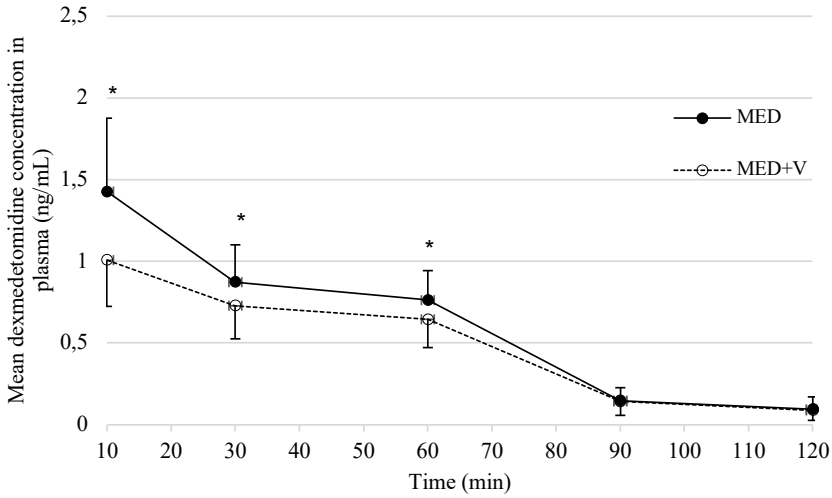

Figure 3. *Mean dexmedetomidine concentration in plasma (study I). Medetomidine hydrochloride (7 µg/kg i.v.) was administered at 0 min alone (MED) or with vatinoxan (140 µg/kg i.v.) (MED+V), followed by a CRI of medetomidine hydrochloride (3.5 µg/kg/h) from 5 min to 65 min. Error bars indicate standard deviation, and * represents a significant difference (p<0.05) between the treatments.*

Table 14. *Mean (± standard deviation) concentration of drugs in plasma and area under the plasma concentration time curve (AUC) (study II). Medetomidine hydrochloride (7 µg/kg i.v.) was administered alone (MED_{GA}) or with vatinoxan (150 µg/kg i.v.) ($MED+V_{GA}$) 10 min before the induction of anesthesia (maintained with an isoflurane and medetomidine CRI at 3.5 µg/kg/h). * Significant difference (p<0.05) between the treatments.*

Drug	Treatment	Concentration in plasma at 1 min before induction (ng/mL)	$AUC_{-1–70\ min}$ (ng•min/mL)	$AUC_{20–70\ min}$ (ng•min/mL)
Dexmedetomidine	MED_{GA}	0.96 ± 0.26	87.4 ± 26.2	-
	$MED+V_{GA}$	0.90 ± 0.26	83.9 ± 17.8	-
Levomedetomidine	MED_{GA}	0.41 ± 0.14	55.5 ± 18.2	-
	$MED+V_{GA}$	0.42 ± 0.17	51.7 ± 13.6	-
Vatinoxan	$MED+V_{GA}$	131 ± 22	5918 ± 620	-
Midazolam	MED_{GA}	-	-	2078 ± 312
	$MED+V_{GA}$	-	-	1890 ± 115
Ketamine	MED_{GA}	-	-	23030 ± 3679
	$MED+V_{GA}$	-	-	18787 ± 2768*

Table 15. *Mean area under the plasma concentration time curve (AUC), and the elimination half-life ($t_{1/2}$) and volume of distribution (Vd) of detomidine, vatinoxan, and hyoscine (study III). The horses received detomidine (20μg/kg i.v.) and, 10 min later, either saline (DET), vatinoxan (150μg/kg i.v.) (DET+V), or hyoscine butylbromide (0.2 mg/kg i.v.) (DET+H). Values with different superscripts [a, b] differ significantly from each other (p<0.05).*

Drug	Treatment	$AUC_{15–90\ min}$ (ng•min/mL)	$t_{1/2}$ (min)	Vd (mL/kg)
Detomidine	DET	546 ± 90	34.5 ± 8.3	1590 ± 109 [a]
	DET+V	509 ± 106	38.6 ± 4.9	1967 ± 435 [a, b]
	DET+H	443 ± 76	36.1 ± 9.3	2026 ± 316 [b]
Vatinoxan	DET+V	9235 ± 2357	53.6 ± 20.5	881 ± 234
Hyoscine	DET+H	11440 ± 2817	21.4 ± 1.5	553 ± 135

6 DISCUSSION

6.1 Cardiopulmonary effects of vatinoxan and hyoscine in standing horses

6.1.1 Systemic vascular resistance and blood pressures

As expected, in studies I and III, medetomidine and detomidine induced vasoconstriction, suggested by the SVRI and SVR, respectively, in which an increase was detected within 15 min from the drug administration. The vasoconstriction after detomidine seemed to last longer than that induced by medetomidine with the doses used in this study, as has been observed previously (Yamashita et al. 2000). The increased SVR translated into an increase in MAP from baseline values with detomidine but not with medetomidine. On the one hand, our findings (with MED) agree with a previous study, in which a medetomidine CRI increased SVR without significantly increasing the MAP from baseline for roughly the same duration as in the present study I (Bettschart-Wolfensberger et al. 1999b). On the other hand, another previous study reported a transient significant increase in MAP from baseline with the same dose of medetomidine as in the current study I (Bryant et al. 1998). This discrepancy in the findings concerning MAP after medetomidine administration could be partially explained by the baseline MAP readings. In the study by Bryant et al. (1998), the baseline MAP was approximately 100 mmHg, whereas in the current study I and the study by Bettschart-Wolfensberger et al. (1999b), the baseline MAP was roughly 130 mmHg. Higher MAP could reflect a possible stress response of the horse standing unmedicated in stocks. Nevertheless, all of these baseline MAP values measured from the carotid artery are within the reported reference range of MAP for a standing horse (94–140 mmHg measured from the facial and transverse facial arteries) (Olsen et al. 2016). In contrast, the increased MAP after detomidine administration in the present study III was clearly above 140 mmHg. Towards the end of the observation period, the SVRI (study I) and SVR (study III) declined along with MAP with MED (study I) and DET (study III). With DET (study III), the decrease in SVR did not reach statistical difference compared to the baseline value during the observation period (90 min). Previously, detomidine has been reported to induce a prolonged hypertensive phase, to the point that a hypotensive phase may remain absent at least for 90–120 minutes (Gasthuys et al. 1990; Yamashita et al. 2000), as was seen in study III as well.

Overall, vatinoxan alleviated and reversed the increased ABP induced by medetomidine (study I) and detomidine (study III), respectively. It is likely that these findings reflect the direct antagonism of vatinoxan to vascular α_2-adrenoceptors and corroborate the findings of previous studies in which vatinoxan was administered simultaneously with medetomidine (Bryant et al. 1998) and romifidine (de Vries et al. 2016) for standing horses. In comparison, hyoscine produced significant hypertension when combined with detomidine. The underlying detomidine-induced vasoconstriction was probably not altered by hyoscine, as no detectable changes in SVR were observed with it (study III), similarly to a previous report (Pimenta et

al. 2011). Therefore, the exacerbated increase in ABP was presumably a consequence of increased HR, i.e. of prevention of the compensatory bradycardia.

Later decreases in ABP after medetomidine administration were also alleviated by vatinoxan in study I. This, however, could be due to an increasing sympathetic activity of the horses receiving vatinoxan (thus recovering from the sedation sooner under the effect of vatinoxan) instead of direct α_2-antagonism. The hypotension induced by α_2-agonists is mainly centrally mediated (Kobinger and Walland 1967; Bousquet and Guertzenstein 1973) and should thus be minimally affected by a peripheral antagonist such as vatinoxan. Conversely, in a previous study, vatinoxan had no effect on the hypotensive phase of medetomidine, even though vatinoxan was administered with a higher dose, 264 µg/kg (Bryant et al. 1998), than in the current study I. In the study by Bryant et al. (1998), however, MAP was measured for only 30 minutes, and medetomidine was administered as a single i.v. bolus, therefore probably rendering the study design incomparable to the present study I.

Despite the MAP decreasing to below the baseline level with vatinoxan in studies I and III, it remained within the reference range (Olsen et al. 2016). Furthermore, no significant changes in SVRI (study I) or SVR (study III) were detected in the presence of vatinoxan. In the study by deVries et al. (2016), vatinoxan alone did not have a hypotensive effect in standing horses, but, similarly to studies I and III, MAP decreased significantly from baseline when romifidine was combined with vatinoxan. As discussed before, the baseline level of MAP may be variable in horses, and comparing the measured MAP values to established reference values in addition to the baseline value is therefore probably advisable. Furthermore, the effects of α_2-agonists in horses (Yamashita et al. 2000) and the effect of vatinoxan on the cardiovascular changes induced by dexmedetomidine in dogs (Honkavaara et al. 2011) are dose-dependent. Therefore, the optimal dose of vatinoxan co-administered with the clinically used doses of α_2-agonists should be determined for horses as well.

The CVP findings correlate with the other hemodynamic findings of the present studies (I and III): the increased CVP after medetomidine (study I) and detomidine (study III) administration was attenuated by vatinoxan. Increased CVP, or right atrial pressure that is considered to be a similar parameter, has been attributed to peripheral and pulmonary vasoconstriction caused by α_2-agonists, resulting in increased preload (Yamashita et al. 2000). Furthermore, α_1- and α_2-adrenoceptors in the venous walls may contribute to venous contraction (Elliott 1997). Conversely, in another study, increased CVP was not observed during a medetomidine CRI (Bettschart-Wolfensberger et al. 1999b). This discrepancy may be partially explained by a higher loading dose used in study I (7 µg/kg) compared to the study by Bettschart-Wolfensberger et al. (1999b) (5 µg/kg). Hyoscine also reduced the CVP after detomidine administration and, in fact, at times the CVP was lower with DET+H than with DET or DET+V (study III). A lower CVP could reflect decreased venous return after hyoscine administration.

6.1.2 Pulmonary vascular resistance and blood pressure

In study III, detomidine induced mild pulmonary artery hypertension, which probably reflected the general vasoconstriction (indicated by increased MAP and SVR) observed with detomidine. This finding is in agreement with previous studies (Wagner et al. 1991; Nyman et al. 2009). The hypertension was alleviated by vatinoxan, notwithstanding that no significant differences were detected in PVR between DET and DET+V. Pulmonary vascular resistance, however, is also influenced by many other factors besides PAP, such as lung volume, right ventricular output, and alveolar and intrapleural pressures (review in Chamarthy et al. 2018). In contrast to DET and DET+V, a transient but significant increase in PAP and PVR was detected with DET+H (study III). The observed changes in cardiac function with DET+H could lead to an increase in PAP, as has been observed in exercising horses in which PAP increases along with the HR and $\dot{Q}t$ (Manohar and Goetz 1999). In contrast to study III, however, PVR actually decreased during heavy exercise due to new pulmonary vascular employment (Manohar and Goetz 1999).

6.1.3 Cardiac function

Medetomidine (study I) and detomidine (study III) expectedly induced bradycardia and reduced $\dot{Q}t$, as has been previously observed in many species, including horses (Gasthuys et al. 1990; Yamashita et al. 2000; Pimenta et al. 2011). Primarily, bradycardia follows the activation of the baroreflex in the face of increased SVR (Flacke et al. 1990), but the centrally reduced sympathetic tone is also a likely contributor. In both studies I and III, α_2-agonist-induced bradycardia was absent in the presence of vatinoxan, probably due to the stable SVR. A similar effect of vatinoxan on HR was reported previously with medetomidine (Bryant et al. 1998), detomidine (Vainionpää et al. 2013), and romifidine (de Vries et al. 2016) in standing horses. In our studies, however, this effect of vatinoxan was firstly shown to last throughout the 1-hour medetomidine CRI and, secondly, to reverse the bradycardia induced by detomidine.

Both vatinoxan and hyoscine reversed the detomidine-induced bradycardia (study III), but considerable hypertension and tachycardia followed hyoscine administration (study III), as has been reported before (Pimenta et al. 2011). Oxygen delivery was maintained but $\dot{V}O_2$ increased, as did LVW with DET+H (study III). Even if these effects of hyoscine were short-lived, they were remarkable (study III). Conversely, by blocking the vasoconstriction, vatinoxan reversed the subsequent bradycardia without marked changes in hemodynamics. Therefore, vatinoxan represents a possible alternative to anticholinergic drugs for the reversal of detomidine-induced bradycardia, with no major cardiovascular or gastrointestinal adverse effects in standing horses. A later study reported on the reversal effects of vatinoxan on α_2-agonist-induced vasoconstriction under general anesthesia, and the results are in concert with ours (Wittenberg-Voges et al. 2018).

6.1.4 Respiratory effects and tissue oxygenation

The RR generally declined with both medetomidine and detomidine in studies I and III, without vatinoxan or hyoscine significantly influencing this decline. Respiratory depression is likely a central effect of α_2-agonists, on which vatinoxan is therefore expected to have a minimal effect.

An inconsistent decrease in PaO_2 was detected with MED in study I, while in study III no changes in PaO_2 caused by detomidine were detected. These findings are in agreement with previous literature that reports variable decreases in PaO_2, along with RR (Gasthuys et al. 1990; Bryant et al. 1998; Bettschart-Wolfensberger et al. 1999b; 2005; Yamashita et al. 2000; Pimenta et al. 2011; Medeiros et al. 2017). We observed no influence of vatinoxan on PaO_2 (studies I and III), whereas a transient decrease in PaO_2 from baseline was observed with DET+H (study III). Conversely, vatinoxan transiently alleviated the decreases in PvO_2 (study I) and $P\bar{v}O_2$ (study III). The changes in venous oxygenation were most evident immediately after drug administration, as were the most evident changes in hemodynamics, similarly to the previous report with romifidine and vatinoxan in standing horses (de Vries et al. 2016). Moreover, a transient, further decrease in $P\bar{v}O_2$ was detected with DET+H, also coinciding with the most evident other hemodynamic changes.

It is plausible that the improved hemodynamics in the presence of vatinoxan contributed to the improved $\dot{D}O_2$ observed with MED+V and DET+V (compared to MED and DET) in the beginning of the observation period in both studies I and III. The marked hemodynamic changes observed with DET+H did not translate into detectable changes in $\dot{D}O_2$, similarly to a previous report (Pimenta et al. 2011). Conversely, neither medetomidine (study I), detomidine (study III), nor vatinoxan produced detectable alterations in $\dot{V}O_2$. In horses receiving hyoscine, however, $\dot{V}O_2$ increased (study III). Therefore, the metabolic rate of tissues could be assumed to be relatively stable with other treatments besides DET+H.

Healthy horses tolerate reduced $\dot{D}O_2$ and hypoxemia to a certain extent quite well. For example, during maximal exercise, PaO_2 can become as low as 58–67 mmHg and PvO_2 as low as 5–15 mmHg (Bayly et al. 1989). Tissues are able to increase their cleavage of oxygen to compensate for reduced $\dot{D}O_2$, and only if PaO_2 or $\dot{D}O_2$ decrease markedly does the $\dot{D}O_2$ become supply-dependent (reviews in Vincent and De Backer 2004; Hubbell and Muir 2015). A recent study evaluated intestinal tissue oxygenation and perfusion, and these remained at the baseline level when arterial oxygen saturation was > 80%, MAP > 51 mmHg, and CI > 40 mL/kg/min. (Hopster et al. 2017a). However, insufficient oxygen supply limits $\dot{V}O_2$ and, consequently, blood lactate levels increase, indicating anaerobic metabolism (Vincent and De Backer 2004). Blood lactate levels were not evaluated in our studies, but as no $\dot{V}O_2$ decreases were detected with detomidine, medetomidine, or vatinoxan, $\dot{D}O_2$ could probably be assumed to remain above critical levels with these treatments.

6.2 Cardiopulmonary effects of vatinoxan in anesthetized horses

Generally, in study II, the effects of vatinoxan on the cardiopulmonary changes induced by medetomidine resembled those seen in standing horses (study I). Some differences were, however, seen. Furthermore, because the cardiopulmonary function is markedly altered by general anesthesia in horses, the effects of vatinoxan are discussed here separately in that context.

6.2.1 Systemic vascular resistance and blood pressures

During general anesthesia, significant hypotension was observed in horses receiving vatinoxan in their premedication (study II). Our observations of SVRI and MAP in studies I and III in standing horses are thus corroborated by study II, with the difference that, during general anesthesia, MAP decreased to below the acceptable limit of 70 mmHg in the presence of vatinoxan. Consequently, significantly more dobutamine was necessary to maintain normotension with MED+V_{GA}.

Hypotension, however, is a common problem in equine anesthesia and can be partially attributed to the hypotensive effects isoflurane (Grosenbaugh and Muir 1998; Hopster et al. 2015). Conversely, a medetomidine CRI has been reported to maintain a higher MAP and SVR during isoflurane anesthesia (Ringer et al. 2007; Risberg et al. 2016), most probably as a result of its vasoconstrictive effect. Previous studies suggested that vatinoxan induces hypotension by antagonizing this vasoconstrictive effect (Pakkanen et al. 2015; Wittenberg-Voges et al. 2018), which is also supported by our finding that SVRI tended to be higher with MED_{GA} than with MED+V_{GA} (study II). However, after 20 min, SVRI no longer differed between the treatments. In contrast, a higher dose of vatinoxan (250 µg/kg i.v.) in another study decreased SVRI for over 1 h in horses anesthetized with isoflurane and a medetomidine CRI (Wittenberg-Voges et al. 2018). The dose-dependent effects of vatinoxan on the cardiovascular changes demonstrated in dogs (Honkavaara et al. 2011) may explain the longer effect of the higher dose of vatinoxan on the SVRI in horses (Wittenberg-Voges et al. 2018; study II). Furthermore, as a positive inotropic drug, dobutamine increases MAP via increasing the $\dot{Q}t$, but via peripheral vasodilation it decreases SVR in anesthetized horses, thus improving perfusion (Dancker et al. 2018). Therefore, dobutamine likely contributed to the observed changes in SVRI and blood pressures (study II). Further dose-response studies on vatinoxan are warranted to limit its hypotensive effect in anaesthetized horses, preferably without confounding factors, such as dobutamine.

6.2.2 Cardiac function

The CI declined significantly more slowly with MED+V_{GA} than MED_{GA}, indicating a better-maintained cardiac performance during anesthesia with MED+V_{GA}, even though the HR measured in connection with the two treatments were comparable. However, the interpretation

of the cardiac performance in study II is confounded by dobutamine. Dobutamine is a vasoactive drug that increases myocardial contractility and $\dot{Q}t$, although with low doses (0.5 μg/kg/min) this effect is not consistently detected in horses (Raisis et al. 2000; Loughran et al. 2017). With MED+V_{GA}, the rate of the dobutamine CRI was, at times, markedly higher than that of MED_{GA} (study II). The higher LVW and SVI that we observed with MED+V_{GA} compared to MED_{GA} (study II) probably reflect increased myocardial contractility, which is an effect of dobutamine. In fact, the CI and SVI were maintained at the baseline level by MED+V_{GA} despite the fact that a decreasing venous return (indicated by decreased CVP) might have decreased the preload. The decreased SVRI with MED+V_{GA} (study II) probably reduced the afterload, contributing to the improved SVI, as seen in dogs (Honkavaara et al. 2011).

6.2.3 Tissue perfusion and oxygenation

Hemoglobin levels increased at 40 min with MED+V_{GA}, concurrently with the PaO_2 concentration (study II), which could be attributed to dobutamine-induced splenic contraction. An increase in hemoglobin concentrations improves the oxygen-carrying capacity of blood, as has been reported before (Loughran et al. 2017). No hypoxemia was observed in any of the horses (study II). Furthermore, $PaCO_2$ remained within a clinically acceptable range, suggesting that all horses were adequately ventilated. There were no differences in venous admixture between the treatments (study II), and the values were comparable with previous studies (Nyman et al. 1990; Briganti et al. 2015), suggesting that neither of the treatments worsened the atelectasis during the observation period. Furthermore, as $PaCO_2$, PVR, or the parameters describing ventilation-perfusion mismatch (venous admixture, VD_{alv}:VT) were not significantly different between the treatments, it seems likely that the differences observed in $\dot{D}O_2I$ between the treatments (study II) were due to alterations in systemic circulation instead of pulmonary function.

As observed in standing horses (studies I and III), $P\bar{v}O_2$ also improved with MED+V_{GA} in comparison to MED_{GA} during general anesthesia (study II). This could result from improved hemodynamics, as previously reported in horses receiving vatinoxan and other α_2-agonists (Pakkanen et al. 2015; de Vries et al. 2016). The severe hypotension observed with MED+V_{GA} responded to dobutamine treatment and, consequently, $\dot{D}O_2I$ was improved compared to MED_{GA}. With the lower SVRI and higher CI with MED+V_{GA}, better perfusion can be assumed, but to confirm this, local blood flow should be measured directly (Raisis et al. 2000; Wittenberg-Voges et al. 2018). In a previous study, vatinoxan actually reduced intestinal perfusion despite an improved CI and SVRI, but the tissue oxygenation was not compromised (Wittenberg-Voges et al. 2018). Recently, however, a lower dose of vatinoxan (130 μg/kg i.v.) and the administration of adjunctive CRIs of both vatinoxan and dexmedetomidine (40 and 7 μg/kg/h i.v., respectively) in isoflurane-anesthetized horses resulted in better hemodynamics and $\dot{D}O_2$ (Neudeck et al. 2018a). Furthermore, the same vatinoxan premedication and CRI also ameliorated the intestinal damage after ischemia and reperfusion applied to the jejunum (Kopp et al. 2018). Using the CRI of vatinoxan, however, may increase the minimal alveolar

concentration of inhalation anesthetics, as has been shown in dogs anesthetized with sevoflurane (Hector et al. 2017).

Lastly, it is noteworthy that the significant decreases in CI and $\dot{D}O_2I$ and increases in O_2ER with MED_{GA} in study II indicate that a medetomidine CRI during general anesthesia can compromise $\dot{D}O_2$, even though clinical cardiovascular parameters (i.e. MAP and HR) may remain acceptable. Because $\dot{V}O_2I$ did not differ between the treatments (study II), it is unlikely that this reduction reached critical levels (Hubbell and Muir 2015), but it may gain more importance in horses with underlying cardiovascular compromise.

6.3 Effect of vatinoxan and hyoscine on intestinal motility in standing horses

Vatinoxan alleviated the decrease in intestinal borborygmi scores throughout the observation period in study I. The scores returned to baseline values in 90 min after administration of concurrent medetomidine and vatinoxan i.v. boluses with MED+V, whereas with MED they were not restored during the observed 120 min. In study III, the intestinal depression induced by detomidine was reversed by vatinoxan in 60 min after detomidine i.v. bolus, while the borborygmi score with DET was not restored in the observed 90 minutes. Furthermore, hyoscine had no detectable effect on the detomidine-induced decrease in the borborygmi score during the observation period (study III). Previously, single i.v. doses of detomidine (30 μg/kg) and medetomidine (10 μg/kg) have been reported to decrease gastrointestinal borborygmi for roughly 2 h and 90 min, respectively (Mama et al. 2009; Grimsrud et al. 2012). The results of studies I and III are consistent with a previous study where vatinoxan prevented the gastrointestinal hypomotility induced by detomidine (Vainionpää et al. 2013). The reversal effects of vatinoxan on gastrointestinal hypomotility, however, have not been reported before. A direct antagonism of vatinoxan at the level of intestinal α_2-adrenoceptors is a plausible explanation for the improved intestinal motility.

In study III no differences were observed in 24-hour fecal output after detomidine sedation between the treatments. This suggests that the depression of intestinal motility after a single dose of detomidine was relatively short-lived in healthy horses and probably did not influence the fecal transit time, similarly to a previous report (Pimenta et al. 2011). The gastrointestinal effects of detomidine or medetomidine may be dose-dependent, like their cardiovascular effects (Yamashita et al. 2000), and, consequently, longer sedations could result in prolonged gastrointestinal depression. Improved gastrointestinal function with vatinoxan could potentially reduce the risk of gastrointestinal disturbances, such as impaction colic, in horses after sedation.

6.4 Effect of vatinoxan on fecal output in anesthetized horses

In study II, fecal output decreased after general anesthesia in all horses, but none of the horses showed signs of gastrointestinal distress. However, decreased fecal output can be a preceding sign of colic after anesthesia, although uncomplicated post-anesthetic ileus also occurs (Little et al. 2001). Vatinoxan did not influence the decreased fecal output in study II, despite the improved gastrointestinal motility in standing horses sedated with medetomidine (study I), detomidine (Vainionpää et al. 2013; study III), or romifidine (de Vries et al. 2016). It is plausible that the gastrointestinal suppression associated with general anesthesia outlasted the effects of a single dose of vatinoxan given in the premedication for the anesthesia.

6.5 Effect of vatinoxan and hyoscine on the quality of sedation

6.5.1 Standing horses

All horses were clinically sedated with medetomidine in study I, but in the presence of vatinoxan the median sedation scores decreased during the first 10 min. The concentration of dexmedetomidine in plasma was reduced with MED+V in study I, which probably explains the decreased sedation scores. In previous studies, the pharmacokinetics of and thus presumably also the tissue exposure to detomidine (Vainionpää et al. 2013) and romifidine (de Vries et al. 2016), have been altered in the presence of vatinoxan in horses. This could likely occur through the changes in hemodynamics, such as lesser vasoconstriction and improved $\dot{Q}t$, leading to increased tissue perfusion as reported before in dogs (Honkavaara et al. 2011; Restitutti et al. 2013). In the previous studies, pharmacokinetic changes resulted in lower concentrations of detomidine and romifidine in plasma and reduced the mean sedation scores (de Vries et al. 2016) and areas under the time-sedation score curves (Vainionpää et al. 2013) with vatinoxan.

Conversely, in the present study III, the level of sedation was not significantly different between DET and DET+V. This is probably because vatinoxan was administered 10 min later than detomidine. The maximal sedative effect of i.v.-administered detomidine is reached in approximately 10 min (Mama et al. 2009). In dogs, simultaneously administered vatinoxan increases the distribution of an i.v. bolus dose of dexmedetomidine mainly during the first 10 min (Honkavaara et al. 2012). In study III, the disposition of detomidine during the first 10 min was not affected by the later administration of vatinoxan and, therefore, no effect on the detomidine concentration in plasma or sedation was detected. Interestingly, in study III, the level of sedation was significantly lower with DET+V compared to DET+H, although no significant difference was detected between the two treatments in the areas under the time-plasma concentration curves. In humans, hyoscine can cause sedation (Ali-Melkkilä et al. 1993), and it may also have potentiated the detomidine-induced sedation in horses. The

potential tendency for hyoscine to enhance sedation and for vatinoxan to reduce sedation could explain the observed difference in sedation scores (study III).

6.5.2 Sedation and the quality of induction before general anesthesia

In contrast to study I and previous reports with other α_2-agonists (Vainionpää et al. 2013; Pakkanen et al. 2015; de Vries et al. 2016), the sedation scores in study II did not differ between the treatments. The results concerning medetomidine concentrations in plasma corroborated this finding, as no difference was detected in them either (study II). Good-quality sedation before inducing general anesthesia is important, as it can smoothen the induction by reducing the amount of circulating catecholamines (England and Clarke 1996). In the presence of vatinoxan, the induction time was faster (study II), as has been reported previously (Pakkanen et al. 2015), likely because of the enhanced cardiovascular function hastening the distribution of ketamine to the brain. The quality score of induction did not significantly differ from that of medetomidine alone (study II). However, with MED+V_{GA}, the poorest induction score was 3, whereas with MED_{GA} all the inductions were scored as 1. Type II (beta) error in sedation and induction scores in study II cannot be excluded.

6.5.3 Recovery from general anesthesia

The reported improvement in the quality of recovery with a medetomidine (Ringer et al. 2007) or dexmedetomidine (Marcilla et al. 2012) CRI has been attributed to its sedative effect, resulting in a longer recumbent time (Ringer et al. 2007; Marcilla et al. 2012). Consequently, horses needed fewer attempts to stand up with a dexmedetomidine CRI compared to sole isoflurane anesthesia (Marcilla et al. 2012). In study II, vatinoxan did not influence the times that the horses spent recumbent after general anesthesia. Therefore, it seems unlikely that vatinoxan, as a single preanesthetic i.v. bolus, would have influenced the recovery quality of these horses.

6.6 Drug concentrations in plasma

We observed differences in the concentrations of dexmedetomidine in plasma (study I), which could be attributed to enhanced hemodynamics altering the distribution and elimination of α_2-agonists in the presence of vatinoxan, as previously reported in dogs (Honkavaara et al. 2012; Restitutti et al. 2017) and horses (Vainionpää et al. 2013; Pakkanen et al. 2015; de Vries et al. 2016). In study III, the area under the plasma concentration time curve of detomidine was not significantly influenced by the later-administered vatinoxan. Conversely, in study I, vatinoxan reduced the areas under the plasma concentration time curves of both enantiomers of medetomidine. Interestingly, before and during general anesthesia (study II), no influence of vatinoxan on the concentration of medetomidine enantiomers in plasma was detected, while

the concentration of ketamine was reduced. Previously, the volume of distribution and clearance of romifidine and detomidine were significantly greater, when the α_2-agonist and vatinoxan were administered simultaneously i.v. in horses (Vainionpää et al. 2013; de Vries et al. 2016). The discrepancy between the results of study III and those of previous studies is likely due to the later administration vatinoxan, as discussed earlier. In studies I and II, the pharmacokinetic parameters were not calculated in as much detail due to the limited number of plasma samples. Therefore, the influence of vatinoxan on the pharmacokinetics of medetomidine during CRI remains to be investigated.

The plasma concentration of ketamine was significantly reduced by vatinoxan in study II during the anesthesia, which could have an influence on the depth of anesthesia. We observed, however, no significant differences in ET_{iso} between MED_{GA} and $MED+V_{GA}$. Conversely, in a previous study, such a difference in ketamine concentration in plasma was not observed in anesthetized horses, albeit the concentrations of detomidine and butorphanol in plasma were reduced by vatinoxan (Pakkanen et al. 2015). Previously, it has been observed that the plasma concentration of α_2-agonists is influenced by simultaneously administered vatinoxan in dogs (Honkavaara et al. 2012) and horses (Vainionpää et al. 2013; Pakkanen et al. 2015; de Vries et al. 2016). Based on the results of study II, it seems that premedication with vatinoxan could alter the concentration of other drugs in plasma (such as ketamine in study II) that are administered later. Furthermore, the improved tissue perfusion with vatinoxan, as demonstrated in dogs (Restitutti et al. 2013), may have resulted in a lower concentration of ketamine in plasma due to an enhanced clearance of the drug.

We mainly measured drug concentrations in plasma from arterial blood obtained from the carotid artery catheter. Arterial blood drug concentrations are higher than those of venous blood during the distribution phase (Fagiolino et al. 2013). Conversely, the venous drug concentration exceeds that of the arteries, when the elimination of the drug is the main process and during the steady state of CRI (Fagiolino et al. 2013). Therefore, collecting samples for plasma drug concentration from the same source during the study would be important when concentrations are compared between the treatments. However, that was not possible in study II before induction, since the artery was not catheterized at that time point due to the risk of bleeding if the catheter had been damaged during induction.

6.7 Limitations of the studies

6.7.1 The horses (studies I–III)

It should be noted that the present studies were performed on healthy animals without surgical or any other stimulus. In dogs, the duration of sedation has been found to be shortened and the antinociceptive action of medetomidine diminished by simultaneously administered vatinoxan (Bennett et al. 2016). The effects of vatinoxan on sedation quality and on the potential analgesic effect of medetomidine and detomidine when a nociceptive stimulus is applied

remain to be investigated in horses. Furthermore, some of the observed effects of medetomidine, detomidine, and vatinoxan on cardiopulmonary and gastrointestinal function may become of greater importance in ill animals, such as horses with colic.

All the horses used in these studies were managed similarly, and their level of physical fitness can be assumed to be similar. Furthermore, the horses were of approximately the same body weight, despite representing two breeds. The age of the horses, however, varied. Ageing has been shown to affect some cardiovascular parameters in horses during exercise (Betros et al. 2002). However, this effect was reported in horses over 20 years old, and whether the same occurs in a resting horse is unknown. The cross-over design of our studies aids in minimizing the effect of inter-horse variation, such as the age.

Finally, the sample size of our studies was small. The sample size was based on a previous, comparable study (Vainionpää et al. 2013) that indicated that a sample size of six would be sufficient in identifying significant differences in clinically relevant cardiopulmonary and gastrointestinal parameters. The cross-over design was also selected in order to increase the power of the study with this sample size.

6.7.2. Lithium dilution method for cardiac output measurement (studies I–III)

The lithium sensor voltage can be affected by several drugs, including medetomidine, dexmedetomidine, detomidine, and ketamine, *in vitro* (Ambrisko et al. 2013). Of these drugs, only ketamine was shown to affect the sensor at concentrations that could be reached with clinically relevant doses. The concentrations of dexmedetomidine (studies I and II), ketamine (study II), and detomidine (study III) in plasma were markedly lower than those that caused relevant bias with the sensor *in vitro* (Ambrisko et al. 2013). Therefore, it appears unlikely that medetomidine, detomidine, or ketamine with the doses used in the present studies would have relevantly affected the results of $\dot{Q}t$ measurement. According to the *in vitro* study by Ambrisko et al. (2013), midazolam is unlikely to interact relevantly with the lithium sensor, but the impact of vatinoxan on the sensor is not known. The data regarding the influence of dobutamine on the lithium sensor is controversial. Comparable results of $\dot{Q}t$ measurements with a lithium dilution and thermodilution methods in horses receiving dobutamine have been reported (Linton et al. 2000, Corley et al. 2002), although others have reported a bias in $\dot{Q}t$ measurement with the lithium dilution method with high doses of dobutamine (Hopster et al. 2017b). Finally, we elected to use the lithium dilution method for measuring $\dot{Q}t$ because it is one of the validated methods in horses (Linton et al. 2000; Corley et al. 2003) and because of the familiarity and availability of the technique.

6.7.3. Medetomidine CRI during general anesthesia (study II)

The design of study II does not allow comparisons between isoflurane anesthesia balanced with medetomidine CRI and isoflurane anesthesia with no adjunctive CRI. A

dexmedetomidine CRI has become popular in horses (Gozalo-Marcilla et al. 2017), despite the concerns about the effect of a dexmedetomidine CRI on cardiovascular function in anesthetized horses (Risberg et al. 2016; Neudeck et al. 2018b). Studies evaluating a dexmedetomidine (Ringer et al. 2007; Valverde et al. 2010) or medetomidine (Marcilla et al. 2012; Gozalo-Marcilla et al. 2013b) CRI in anesthetized horses report that its suppressing effects on cardiovascular function are limited. Nevertheless, it remains to be determined how the investigated protocols of study II compare with sole isoflurane anesthesia.

6.7.4. The impact of dobutamine (study II)

As discussed earlier, the administration of dobutamine is likely to confound the interpretation of some of the cardiopulmonary parameters during general anesthesia in study II. Performing cardiopulmonary measurements prior to the administration of dobutamine during general anesthesia could have aided in overcoming this limitation. However, the well-being of the horses during anesthesia could have been compromised if the support of the cardiovascular status by dobutamine had been delayed. Lastly, as the concentrations of dobutamine in plasma were not measured (due to practical reasons), it remains unknown whether vatinoxan had an influence on them.

6.8. Clinical implications and future considerations

Overall, vatinoxan improved the cardiopulmonary function in healthy, unstimulated horses sedated with a medetomidine CRI, although the cardiopulmonary changes during a medetomidine CRI in these horses could be judged clinically minor. Nevertheless, the improved oxygenation and tissue perfusion in the presence of vatinoxan may become clinically important in sedation protocols for longer surgical procedures or in horses with compromised cardiopulmonary function. Furthermore, the effects of vatinoxan on cardiopulmonary function during longer sedation with a CRI of other commonly used α_2-agonists, such as detomidine, and in stimulated horses remains to be investigated.

The improved intestinal motility induced by vatinoxan during sedation with a medetomidine CRI is clinically relevant. As many surgical procedures, such as dental operations, are increasingly performed in standing horses and the procedures may last hours, the suppression of intestinal motility by α_2-agonists is a concern. Vatinoxan could aid in preventing post-sedation colic due to intestinal hypomotility in horses sedated for longer periods of time.

Marked hypotension remains a concern when vatinoxan is administered in association with general anesthesia in horses despite the fact that the hypotension observed in study II responded to dobutamine treatment. Therefore, further research on vatinoxan in anesthetized horses is warranted before it could be used during or before general anesthesia. A future target of research in this regard could be elucidating an optimal dose of vatinoxan with commonly used α_2-agonists in horses before general anesthesia. Also, recently published results on the

administration of vatinoxan as a CRI during anesthesia (Kopp et al. 2018; Neudeck et al. 2018a) are encouraging and, thus, warrant more investigation.

In study III vatinoxan reversed the bradycardia—and, importantly, the other cardiopulmonary and gastrointestinal effects—induced by detomidine more effectively than the anticholinergic drug hyoscine. Therefore, vatinoxan could be an alternative drug to reverse α_2-agonist-induced bradycardia in standing horses. The hypotensive effects of vatinoxan during general anesthesia should be resolved, however, before these results can be extrapolated to the treatment of bradycardia in anesthetized horse.

7 CONCLUSIONS

1. Heart rate decreased significantly less, and the SVRI and CI were maintained at the baseline level during a medetomidine CRI when vatinoxan was administered i.v. with a loading dose of medetomidine. The MAP decreased significantly more from the baseline with vatinoxan but remained within the reference range. Thus, vatinoxan improved the hemodynamics during a CRI of medetomidine in standing horses. (I)

2. Premedication with vatinoxan before general anesthesia induced hypotension in horses anesthetized with isoflurane and a CRI of medetomidine. Even though the HR and ABP remained clinically acceptable in horses premedicated with medetomidine without vatinoxan, cardiac performance and $\dot{D}O_2I$ were decreased compared to horses receiving vatinoxan. (II)

3. Vatinoxan, administered after detomidine, reversed or attenuated the adverse cardiopulmonary effects of detomidine, while hyoscine induced marked tachycardia and exacerbated the detomidine-induced hypertension in standing horses. Thus, vatinoxan could be a suitable alternative to reverse the α_2-agonist-induced bradycardia because it reverses the primary hemodynamic changes instead of solely affecting the subsequent bradycardia. (III)

4. Vatinoxan improved the intestinal hypomotility induced by detomidine and a CRI of medetomidine but not the hypomotility associated with general anesthesia. It is plausible that the hypomotility associated with general anesthesia outlasted the effects of a single dose of vatinoxan, whereas with shorter standing sedations, the effects of a single dose of vatinoxan were of sufficient duration. (I–III)

5. Vatinoxan did not affect the level of sedation or the concentration of detomidine or medetomidine in plasma when administered after detomidine i.v. or simultaneously with a single bolus of medetomidine before general anesthesia. When administered simultaneously with a loading dose of medetomidine, vatinoxan reduced the level of sedation during the first 10 min of a CRI of medetomidine. (I–III)

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REFERENCES

Adam M, Huuskonen V, Raekallio MR et al. (2018a) Cardiopulmonary effects of vatinoxan in sevoflurane-anaesthetised sheep receiving dexmedetomidine. *The Veterinary Journal* 238, 63-69.

Adam M, Raekallio MR, Keskitalo T et al. (2018b) 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.

Ali-Melkkilä T, Kanto J, Iisalo E. (1993) Pharmacokinetics and related pharmacodynamics of anticholinergic drugs. *Acta Anaesthesiologica Scandinavica* 37, 633-642.

Alitalo I, Vainio O, Kaartinen L et al. (1986) Cardiac effects of atropine premedication in horses sedated with detomidine. *Acta Veterinaria Scandinavica Supplementum* 82, 131-136.

Ambrisko TD, Kabes R, Moens Y. (2013) Influence of drugs on the response characteristics of the LiDCO sensor: an in vitro study. *British Journal of Anaesthesia* 110, 305-310.

Andersen MS, Clark L, Dyson SJ et al. (2006) Risk factors for colic in horses after general anaesthesia for MRI or nonabdominal surgery: absence of evidence of effect from perianaesthetic morphine. *Equine Veterinary Journal* 38, 368-374.

Auckburally A, Nyman G. (2017) Review of hypoxaemia in anaesthetized horses: predisposing factors, consequences and management. *Veterinary anaesthesia and analgesia* 44, 397-408.

Bayly WM, Hodgson DR, Schulz DA et al. (1989) Exercise-induced hypercapnia in the horse. *Journal Applied Physiology* 67, 1958-1966.

Bennett RC, Salla KM, Raekallio MR et al. (2016) Effects of MK-467 on the antinociceptive and sedative actions and pharmacokinetics of medetomidine in dogs. *Journal of Veterinary Pharmacology and Therapeutics* 39, 336-343.

Betros CL, McKeever KH, Kearns CF et al. (2002) Effects of ageing and training on maximal heart rate and VO2max. *Equine Veterinary Journal* 34, 100-105.

Bettembourg V, Dulgheriu D, Haga HA. (2019) Plasma concentrations at two dexmedetomidine constant rate infusions in isoflurane anaesthetized horses: a clinical study. *Veterinary Anaesthesia and Analgesia* 46, 627-635.

Bettschart-Wolfensberger R, Clarke KW, Vainio O et al. (1999a) Pharmacokinetics of medetomidine in ponies and elaboration of a medetomidine infusion regime which provides a constant level of sedation. *Research in Veterinary Science* 67, 41-46.

Bettschart-Wolfensberger R, Bettschart RW, Vainio O et al. (1999b) Cardiopulmonary effects of a two hour medetomidine infusion and its antagonism by atipamezole in horses and ponies. *Veterinary Anaesthesia and Analgesia* 26, 8-12.

Bettschart-Wolfensberger R, Jaggin-Schmucker N, Lendl C et al. (2001) Minimal alveolar concentration of desflurane in combination with an infusion of medetomidine for the anaesthesia of ponies. *Veterinary Record* 148, 264-267.

Bettschart-Wolfensberger R, Freeman SL, Bowen IM et al. (2005) Cardiopulmonary effects and pharmacokinetics of i.v. dexmedetomidine in ponies. *Equine Veterinary Journal* 37, 60-64.

Bettschart-Wolfensberger R, Larenza MP. (2007) Balanced anesthesia in the equine. *Clinical Techniques in Equine Practice* 6, 104-110.

Bidwell LA, Bramlage LR, Rood WA. (2007) Equine perioperative fatalities associated with general anaesthesia at a private practice – a retrospective case series. *Veterinary Anaesthesia and Analgesia* 34, 23-30.

Blandizzi C, Doda M, Tarkovacs G et al. (1991) Functional evidence that acetylcholine release from Auerbach's plexus of guinea-pig ileum is modulated by alpha 2A-adrenoceptor subtype. *European Journal of Pharmacology* 205, 311-313.

Bousquet P, Guertzenstein PG. (1973) Localization of the central cardiovascular action of clonidine. *British Journal of Pharmacology* 49, 573-579.

Bowen IM, Marr CM, Chester AH et al. (2004) In-vitro contraction of the equine aortic valve. *The Journal of Heart Valve Disease* 13, 593-599.

Boyd CJ, McDonell WN, Valliant A. (1991) Comparative hemodynamic effects of halothane and halothane-acepromazine at equipotent doses in dogs. *Canadian Journal of Veterinary Research* 55, 107-112.

Briganti A, Portela DA, Grasso S et al. (2015) Accuracy of different oxygenation indices in estimating intrapulmonary shunting at increasing infusion rates of dobutamine in horses under general anaesthesia. *The Veterinary Journal* 204, 351-356.

Brightman MW, Reese TS. (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. *The Journal of Cell Biology* 40, 648-677.

Brodde OE, Bruck H, Leineweber K et al. (2001) Presence, distribution and physiological function of adrenergic and muscarinic receptor subtypes in the human heart. *Basic Research in Cardiology* 96, 528-538.

Browning KN, Travagli RA. (2014) Central nervous system control of gastrointestinal motility and secretion and modulation of gastrointestinal functions. *Comprehensive Physiology* 4, 1339-1368.

Bryant CE, Clarke KW, Thompson J. (1996) Cardiopulmonary effects of medetomidine in sheep and in ponies. *Research in Veterinary Science* 60, 267-271.

Bryant CE, Thompson J, Clarke KW. (1998) Characterisation of the cardiovascular pharmacology of medetomidine in the horse and sheep. *Research in Veterinary Science* 65, 149-154.

Buchner HH, Kubber P, Zohmann E et al. (1999) Sedation and antisedation as tools in equine lameness examination. *Equine Veterinary Journal Supplement* 30, 227-230.

Buhl R, Ersboll AK, Larsen NH et al. (2007) The effects of detomidine, romifidine or acepromazine on echocardiographic measurements and cardiac function in normal horses. *Veterinary Anaesthesia and Analgesia* 34, 1-8.

Burnstock G. (2009) Autonomic neurotransmission: 60 years since sir Henry Dale. *Annual Review of Pharmacology and Toxicology* 49, 1-30.

Bylund DB, Eikenberg DC, Hieble JP et al. (1994) International Union of Pharmacology nomenclature of adrenoceptors. *Pharmacological Reviews* 46, 121-136.

Carroll GL, Matthews NS, Hartsfield SM et al. (1997) The effect of detomidine and its antagonism with tolazoline on stress-related hormones, metabolites, physiologic responses, and behavior in awake ponies. *Veterinary Surgery* 26, 69-77.

Casbeer HC, Knych HK. (2013) Pharmacokinetics and pharmacodynamic effects of tolazoline following intravenous administration to horses. *The Veterinary Journal* 196, 504-509.

Caulfield MP. (1993) Muscarinic receptors—characterization, coupling and function. *Pharmacology & Therapeutics* 58, 319-379.

Chamarthy MR, Kandathil A, Kalva SP. (2018) Pulmonary vascular pathophysiology. *Cardiovascular Diagnosis and Therapy* 8, 208-213.

Clarke KW, Hall LW. (1969) "Xylazine"--a new sedative for horses and cattle. *Veterinary Record* 85, 512-517.

Clarke KW, Taylor PM. (1986) Detomidine: A new sedative for horses. *Equine Veterinary Journal* 18, 366-370.

Clerbaux T, Gustin P, Detry B et al. (1993) Comparative study of the oxyhaemoglobin dissociation curve of four mammals: man, dog, horse and cattle. *Comparative Biochemistry and Physiology* 106, 687-694.

Clineschmidt BV, Pettibone DJ, Lotti VJ et al. (1988) A peripherally acting alpha-2 adrenoceptor antagonist: L-659,066. *The Journal of Pharmacology and Experimental Therapeutics* 245, 32-40.

Colucci R, Blandizzi C, Carignani D et al. (1998) Effects of imidazoline derivatives on cholinergic motility in guinea-pig ileum: involvement of presynaptic α2-adrenoceptors or imidazoline receptors? *Naunyn-Schmiedeberg's Archives of Pharmacology* 357, 682-691.

Corley K, Donaldson LL, Furr MO. (2002) Comparison of lithium dilution and thermodilution cardiac output measurements in anaesthetised neonatal foals. *Equine Veterinary Journal* 34, 598-601.

Corley KT, Donaldson LL, Durando MM et al. (2003) Cardiac output technologies with special reference to the horse. *Journal of Veterinary Internal Medicine* 17, 262-272.

Costa-Farré C, Prades M, Ribera T et al. (2014) Does intraoperative low arterial partial pressure of oxygen increase the risk of surgical site infection following emergency exploratory laparotomy in horses? *The Veterinary Journal* 200, 175-180.

Dancker C, Hopster K, Rohn K et al. (2018) Effects of dobutamine, dopamine, phenylephrine and noradrenaline on systemic haemodynamics and intestinal perfusion in isoflurane anaesthetised horses. *Equine Veterinary Journal* 50, 104-110.

Daunt DA, Dunlop CI, Chapman PL et al. (1993) Cardiopulmonary and behavioral responses to computer-driven infusion of detomidine in standing horses. *American Journal Veterinary Research* 54, 2075-2082.

Daunt DA, Steffey EP. (2002) Alpha-2 adrenergic agonists as analgesics in horses. *Veterinary Clinics of North America: Equine Practice* 18, 46, vi.

De Ponti F, Giaroni C, Cosentino M et al. (1996) Adrenergic mechanisms in the control of gastrointestinal motility: from basic science to clinical applications. *Pharmacology & Therapeutics* 69, 59-78.

Devisscher L, Schauvliege S, Dewulf J et al. (2010) Romifidine as a constant rate infusion in isoflurane anaesthetized horses: a clinical study. *Veterinary Anaesthesia and Analgesia* 37, 425-433.

de Vries A, Pakkanen SA, Raekallio MR et al. (2016) Clinical effects and pharmacokinetic variables of romifidine and the peripheral α_2-adrenoceptor antagonist MK-467 in horses. *Veterinary Anaesthesia and Analgesia* 43, 511-610.

Di Concetto S, Michael Archer R, Sigurdsson SF et al. (2007) Atipamezole in the management of detomidine overdose in a pony. *Veterinary Anaesthesia and Analgesia* 34, 67-69.

DiMaio Knych HK, Stanley SD. (2011) Pharmacokinetics and pharmacodynamics of detomidine following sublingual administration to horses. *American Journal of Veterinary Research* 72, 1378-1385.

DiMaio Knych HK, Covarrubias V, Steffey EP. (2012) Effect of yohimbine on detomidine induced changes in behavior, cardiac and blood parameters in the horse. *Veterinary Anaesthesia and Analgesia* 39, 574-583.

Dirikolu M, McFadde ET, Ely KJ. (2006) Clonidine in Horses: Identification, Detection, and Clinical Pharmacology. *Veterinary Therapeutics* 7, 141-155.

Dogrul A, Coskun I, Uzbay T. (2006) The contribution of alpha-1 and alpha-2 adrenoceptors in peripheral imidazoline and adrenoceptor agonist-induced nociception. *Anesthesia & Analgesia* 103, 471-477.

Ducharme NG, Fubini SL. (1983) Gastrointestinal complications associated with the use of atropine in horses. *Journal of American Veterinary Medical Association* 182, 229-231.

Dugdale AHA, Obhrai J, Cripps PJ. (2016) Twenty years later: a single-centre, repeat retrospective analysis of equine perioperative mortality and investigation of recovery quality. *Veterinary Anaesthesia and Analgesia* 43, 171-178.

Dugdale AH, Taylor PM. (2016) Equine anaesthesia-associated mortality: where are we now? *Veterinary anaesthesia and analgesia* 43, 242-255.

Dutta S, Lal R, Karol MD et al. (2000) Influence of cardiac output on dexmedetomidine pharmacokinetics. *Journal of Pharmaceutical Sciences* 89, 519-527.

Dyrna F, Hanske S, Krueger M et al. (2013) The blood-brain barrier. *Journal of Neuroimmune Pharmacology* 8, 763-773.

Elfenbein JR, Sanchez LC, Robertson SA et al. (2009) Effect of detomidine on visceral and somatic nociception and duodenal motility in conscious adult horses. *Veterinary Anaesthesia and Analgesia* 36, 162-172.

Elliott J. (1997) Alpha-adrenoceptors in equine digital veins: Evidence for the presence of both $alpha_1$ and $alpha_2$-receptors mediating vasoconstriction. *Journal of Veterinary Pharmacology and Therapeutics* 20, 308-317.

England GC, Clarke KW. (1996) $Alpha_2$ adrenoceptor agonists in the horse--a review. *British Veterinary Journal* 152, 641-657.

Enouri SS, Kerr CL, McDonell WN et al. (2008) Effects of a peripheral α_2 adrenergic-receptor antagonist on the hemodynamic changes induced by medetomidine administration in conscious dogs. *American Journal of Veterinary Research* 69, 728-736.

Ernsberger P, Friedman JE, Koletsky RJ. (1997) The I_1-imidazoline receptor: from binding site to therapeutic target in cardiovascular disease. *Journal of Hypertension Supplement* 15, S9-23.

Fagiolino P, Vázquez M, Eiraldi R. (2013) Clearance and bioavailability study through arterio-venous drug concentrations relationship. *European Journal of Pharmaceutical Sciences* 48, 825-829.

Fargeas MJ, Fioramonti J, Bueno L. (1986) Central α_2-adrenergic control of the pattern of small intestinal motility in rats. *Gastroenterology* 91, 1470-1475.

Flacke JW, Flacke WE, Bloor BC et al. (1990) Hemodynamic effects of dexmedetomidine, an α_2-adrenergic agonist, in autonomically denervated dogs. *Journal of Cardiovascular Pharmacology* 16, 616-623.

Flacke WE, Flacke JW, Blow KD et al. (1992) Effect of dexmedetomidine, an α_2-adrenergic agonist, in the isolated heart. *Journal of Cardiothoracic and Vascular Anesthesia* 6, 418-423.

Fornai F, Blandizzi C, Del Tacca M. (1990) Central alpha-2 adrenoceptors regulate central and peripheral functions. *Pharmacological research* 22, 541-554.

Furness JB. (2012) The enteric nervous system and neurogastroenterology. *Nature reviews Gastroenterology & hepatology* 9, 286-294.

Gasthuys F, De Moor A, Parmentier D. (1990) Haemodynamic changes during sedation in ponies. *Veterinary Research Communications* 14, 309-327.

Geimer TR, Ekstrom PM, Ludders JW et al. (1995) Haemodynamic effects of hyoscine-N-butylbromide in ponies. *Journal of Veterinary Pharmacology and Therapeutics* 18, 13-16.

Gertler R, Brown HC, Mitchell DH et al. (2001) Dexmedetomidine: a novel sedative-analgesic agent. *Proceedings of the Baylor University Medical Center* 14, 13-21.

Gil DW, Cheevers CV, Kedzie KM et al. (2009) α-1-Adrenergic receptor agonist activity of clinical α-adrenergic receptor agonists interferes with α-2-mediated analgesia. *Anesthesiology* 110, 401-407.

Gozalo-Marcilla M, Hopster K, Gasthuys F et al. (2013a) Effects of a constant-rate infusion of dexmedetomidine on the minimal alveolar concentration of sevoflurane in ponies. *Equine Veterinary Journal* 45, 204-208.

Gozalo-Marcilla M, Steblaj B, Schauvliege S et al. (2013b) Comparison of the influence of two different constant-rate infusions (dexmedetomidine versus morphine) on anaesthetic requirements, cardiopulmonary function and recovery quality in isoflurane anaesthetized horses. *Research in Veterinary Science* 95, 1186-1194.

Gozalo-Marcilla M, Gasthuys F, Schauvliege S. (2014) Partial intravenous anaesthesia in the horse: review of intravenous agents used to supplement equine inhalation anaesthesia. Part 1: lidocaine and ketamine. *Veterinary Anaesthesia and Analgesia* 41, 335-345.

Gozalo-Marcilla M, Gasthuys F, Schauvliege S. (2015) Partial intravenous anaesthesia in the horse: a review of intravenous agents used to supplement equine inhalation anaesthesia. Part 2: opioids and alpha-2 adrenoceptor agonists. *Veterinary Anaesthesia and Analgesia* 42, 1-16.

Gozalo-Marcilla M, Gasthuys F, Luna SPL et al. (2017) Is there a place for dexmedetomidine in equine anaesthesia and analgesia? A systematic review (2005-2017). *Journal of Veterinary Pharmacology and Therapeutics* 41, 205-217.

Gozalo-Marcilla M, Luna SPL, Moreina Da Silva R et al. (2019a) Characterisation of the in vivo interactions between detomidine and methadone in horses: Pharmacokinetic and pharmacodynamic modelling. *Equine Veterinary Journal* 51, 517-529.

Gozalo-Marcilla M, De Oliveira AM, Fonseca MW et al. (2019b) Sedative and antinociceptive effects of different detomidine constant rate infusions, with or without methadone in standing horses. *Equine Veterinary Journal* 51, 530-536.

Grimsrud KN, Mama KR, Thomasy SM et al. (2009) Pharmacokinetics of detomidine and its metabolites following intravenous and intramuscular administration in horses. *Equine Veterinary Journal* 41, 361-365.

Grimsrud KN, Mama KR, Steffey EP et al. (2012) Pharmacokinetics and pharmacodynamics of intravenous medetomidine in the horse. *Veterinary Anaesthesia and Analgesia* 39, 38-48.

Grosenbaugh DA, Muir WW. (1998) Cardiorespiratory effects of sevoflurane, isoflurane, and halothane anesthesia in horses. *American Journal of Veterinary Research* 59, 101-106.

Guimarães S, Moura D. (2001) Vascular adrenoceptors: an update. *Pharmacological reviews* 53, 319-356.

Hallowell GD, Corley KT. (2005) Use of lithium dilution and pulse contour analysis cardiac output determination in anaesthetized horses: a clinical evaluation. *Veterinary Anaesthesia and Analgesia* 32, 201-211.

Hamlin RL, Klepinger WL, Gilpin KW et al. (1972) Autonomic control of heart rate in the horse. *American Journal of Physiology* 222, 976-978.

Hardman JG, Aitkenhead AR. (2003) Estimating alveolar dead space from the arterial to end-tidal CO(2) gradient: a modeling analysis. *Anesthesia & Analgesia* 97, 1846-1851.

Haskins S, Pascoe PJ, Ilkiw JE et al. (2005) Reference cardiopulmonary values in normal dogs. *Comparative Medicine* 55, 156-161.

Head GA, Mayorov DN. (2006) Imidazoline receptors, novel agents and therapeutic potential. *Cardiovascular & Hematological Agents in Medicinal Chemistry* 4, 17-32.

Hector RC, Rezende ML, Mama KR et al. (2017) Effects of constant rate infusions of dexmedetomidine or MK-467 on the minimum alveolar concentration of sevoflurane in dogs. *Veterinary Anaesthesia and Analgesia* 44, 755-765.

Hikasa Y, Masuda K, Asakura Y et al. (2013) Identification and characterization of platelet α_2-adrenoceptors and imidazoline receptors in rats, rabbits, cats, dogs, cattle, and horses. *The European Journal of Pharmacology* 720, 363-375.

Hobo S, Aida H, Yoshida K. (1995) Assessment of the sedative effect of medetomidine and determination of its optimal dose in thoroughbred horses. *Journal of Veterinary Medical Science* 57, 507-510.

Honkavaara JM, Raekallio MR, Kuusela EK et al. (2008) The effects of L-659,066, a peripheral α2-adrenoceptor antagonist, on dexmedetomidine-induced sedation and bradycardia in dogs. *Veterinary Anaesthesia and Analgesia* 35, 409-413.

Honkavaara JM, Restitutti F, Raekallio MR et al. (2011) The effects of increasing doses of MK-467, a peripheral alpha$_2$-adrenergic receptor antagonist, on the cardiopulmonary effects of intravenous dexmedetomidine in conscious dogs. *Journal of Veterinary Pharmacology and Therapeutics* 34, 332-337.

Honkavaara J, Restitutti F, Raekallio M et al. (2012) Influence of MK-467, a peripherally acting α_2-adrenoceptor antagonist on the disposition of intravenous dexmedetomidine in dogs. *Drug Metabolism and Disposition* 40, 445-449.

Honkavaara J, Pypendop B, Turunen H et al. (2017a) The effect of MK-467, a peripheral α_2-adrenoceptor antagonist, on dexmedetomidine-induced sedation and bradycardia after intravenous administration in conscious cats. *Veterinary Anaesthesia and Analgesia* 44, 42-51.

Honkavaara J, Pypendop B, Ilkiw J. (2017b) The impact of MK-467 on sedation, heart rate and arterial blood pressure after intramuscular coadministration with dexmedetomidine in conscious cats. *Veterinary Anaesthesia and Analgesia* 44, 811-822.

Honkavaara JM, Raekallio MR, Syrjä PM et al. (in press) Concentrations of medetomidine enantiomers and vatinoxan, an α_2-adrenoceptor antagonist, in plasma and central nervous tissue after intravenous coadministration in dogs. *Veterinary Anaesthesia and Analgesia* https://doi.org/10.1016/j.vaa.2019.07.004.

Hopster K, Ambrisko TD, Kästner SB. (2017) Influence of catecholamines at different dosages on the function of the LiDCO sensor in isoflurane anesthetized horses. *Journal of Veterinary Emergency and Critical Care* 27, 651-657.

Hopster K, Hopster-Iversen C, Geburek F et al. (2015) Temporal and concentration effects of isoflurane anaesthesia on intestinal tissue oxygenation and perfusion in horses. *The Veterinary Journal* 205, 62-68.

Hopster K, Wittenberg-Voges L, Geburek F et al. (2017a) Effects of controlled hypoxemia or hypovolemia on global and intestinal oxygenation and perfusion in isoflurane anesthetized horses receiving an alpha-2-agonist infusion. *BMC Veterinary Research* 13, 361.

Hubbell JA, Muir WW. (2015) Oxygenation, oxygen delivery and anaesthesia in the horse. *Equine Veterinary Journal* 47, 25-35.

Huwyler J, Fricker G, Török M et al. (1997) Transport of clonidine across cultured brain microvessel endothelial cells. *The Journal of Pharmacology and Experimental Therapeutics* 282, 81-85.

Johnston GM, Eastment JK, Wood J et al. (2002) The confidential enquiry into perioperative equine fatalities (CEPEF): mortality results of Phases 1 and 2. *Veterinary Anaesthesia and Analgesia* 29, 159-170.

Jöchle W, Hamm D. (1986) Sedation and analgesia with Domosedan (detomidine hydrochloride) in horses: dose response studies on efficacy and its duration. *Acta Veterinaria Scandinavica Supplementum* 82, 69-84.

Kalchofner KS, Ringer SK, Boller J et al. (2006) Clinical assessment of anesthesia with isoflurane and medetomidine in 300 equidae. *Pferdeheilkunde* 22, 301-308.

Kallio-Kujala IJ, Raekallio MR, Honkavaara J et al. (2018) Peripheral α_2-adrenoceptor antagonism affects the absorption of intramuscularly coadministered drugs. *Veterinary anaesthesia and analgesia* 45, 405-413.

Kaukinen H, Aspegren J, Hyyppä S et al. (2011) Bioavailability of detomidine administered sublingually to horses as an oromucosal gel. *Journal of Veterinary Pharmacology and Therapeutics* 34, 76-81.

Knaus AE, Muthig V, Schickinger S et al. (2007) α_2-adrenoceptor subtypes—unexpected functions for receptors and ligands derived from gene-targeted mouse models. *Neurochemistry International* 51, 277-281.

Knych HK, Stanley SD. (2014) Effects of three antagonists on selected pharmacodynamic effects of sublingually administered detomidine in the horse. *Veterinary anaesthesia and analgesia* 41, 36-47.

Kobinger W, Walland A. (1967) Investigations into the mechanism of the hypotensive effect of 2-(2,6-dichlorphenylamino)-2-imidazoline-HCl. *European Journal of Pharm*acology 2, 155-162.

Kollias-Baker CA, Court MH, Williams LL. (1993) Influence of yohimbine and tolazoline on the cardiovascular, respiratory, and sedative effects of xylazine in the horse. *Journal of Veterinary Pharmacology and Therapeutics* 16, 350-358.

Kopp V, Neudeck S, Pfarrer C et al. (2018) Protective effects of dexmedetomidine-vatinoxan versus dexmedetomidine alone on intestinal ischemia-reperfusion injury in horses under general anesthesia. *In World Congress of Veterinary Anesthesiology Proceedings 2018 Venice*.

Lawrence CJ, Prinzen FW, de Lange S. (1996) The effect of dexmedetomidine on the balance of myocardial energy requirement and oxygen supply and demand. *Anesthesia & Analgesia* 82, 544-550.

Levey AI. (1993) Immunological localization of m1–m5 muscarinic acetylcholine receptors in peripheral tissues and brain. *Life Sciences* 52, 441-448.

Li J, Zhang Y. (2011) Imidazoline I_2 receptors: target for new analgesics? *European Journal of Pharmacology* 658, 49-56.

Lindsay WA, Robinson GM, Brunson DB et al. (1989) Induction of equine postanesthetic myositis after halothane-induced hypotension. *American Journal of Veterinary Research* 50, 404-410.

Link RE, Desai K, Stevens ME et al. (1996) Cardiovascular regulation in mice lacking α_2-adrenergic receptor subtypes b and c. *Science* 273, 803-805.

Linton RA, Young LE, Marlin DJ et al. (2000) Cardiac output measured by lithium dilution, thermodilution, and transesophageal Doppler echocardiography in anesthetized horses. *American Journal of Veterinary Research* 61, 731-737.

Little D, Redding WR, Blikslager AT. (2001) Risk factors for reduced postoperative fecal output in horses: 37 cases (1997-1998). *Journal of American Veterinary Medical Association* 218, 414-420.

Loughran CM, Raisis AL, Hosgood G et al. (2017) The effect of dobutamine and bolus crystalloid fluids on the cardiovascular function of isoflurane-anaesthetised horses. *Equine Veterinary Journal* 49, 369-374.

Lowe JE, Hilfiger J. (1986) Analgesic and sedative effects of detomidine compared to xylazine in a colic model using iv and im routes of administration. *Acta Veterinaria Scandinavica.Supplementum* 82, 85-95.

MacMillan LB, Hein L, Smith MS et al. (1996) Central hypotensive effects of the α_2-adrenergic receptor subtype. *Science* 273, 801-803.

Malone ED, Brown DR, Trent AM et al. (1996) Influence of adrenergic and cholinergic mediators on the equine jejunum in vitro. *American Journal of Veterinary Research* 57, 884-890.

Maltsev AV, Kokoz YM, Evdokimovskii EV et al. (2014) Alpha-2 adrenoceptors and imidazoline receptors in cardiomyocytes mediate counterbalancing effect of agmatine on NO synthesis and intracellular calcium handling. *Journal of Molecular and Cellular Cardiology* 68, 66-74.

Mama KR, Grimsrud K, Snell T et al. (2009) Plasma concentrations, behavioural and physiological effects following intravenous and intramuscular detomidine in horses. *Equine Veterinary Journal* 41, 772-777.

Manohar M, Goetz TE. (1999) Pulmonary vascular resistance of horses decreases with moderate exercise and remains unchanged as workload is increased to maximal exercise. *Equine Veterinary Journal Supplement* 30, 117-121.

Marcilla MG, Schauvliege S, Duchateau L et al. (2010) Cardiopulmonary effects of two constant rate infusions of dexmedetomidine in isoflurane anaesthetized ponies. *Veterinary Anaesthesia and Analgesia* 37, 311-321.

Marcilla MG, Schauvliege S, Segaert S et al. (2012) Influence of a constant rate infusion of dexmedetomidine on cardiopulmonary function and recovery quality in isoflurane anaesthetized horses. *Veterinary Anaesthesia and Analgesia* 39, 49-58.

Marly-Voquer C, Schwarzwald CC, Bettschart-Wolfensberger R. (2016) The use of dexmedetomidine continuous rate infusion for horses undergoing transvenous electrical cardioversion--A case series. *Canadian Veterinary Journal* 57, 70-75.

Marques JA, Teixeira Neto FJ, Campebell RC et al. (1998) Effects of hyoscine-N-butylbromide given before romifidine in horses. *Veterinary Record* 142, 166-168.

Marti M, Mevissen M, Althaus H et al. (2005) In vitro effects of bethanechol on equine gastrointestinal contractility and functional characterization of involved muscarinic receptor subtypes. *Journal of Veterinary Pharmacology and Therapeutics* 28, 565-574.

Maugeri S, Ferrè JP, Intorre L et al. (1994) Effects of medetomidine on intestinal and colonic motility in the dog. *Journal of Veterinary Pharmacology and Therapeutics* 17, 148-154.

Medeiros LQ, Gozalo-Marcilla M, Taylor PM et al. (2017) Sedative and cardiopulmonary effects of dexmedetomidine infusions randomly receiving, or not, butorphanol in standing horses. *Veterinary Record* 181, 402.

Menzies MPL, Ringer SK, Conrot A et al. (2016) Cardiopulmonary effects and anaesthesia recovery quality in horses anaesthetized with isoflurane and low-dose S-ketamine or medetomidine infusions. *Veterinary Anaesthesia and Analgesia* 43, 623-634.

Merritt AM, Burrow JA, Hartless CS. (1998) Effect of xylazine, detomidine, and a combination of xylazine and butorphanol on equine duodenal motility. *American Journal of Veterinary Research* 59, 619-623.

Moens Y, Lanz F, Doherr Mg et al. (2003) A comparison of the antinociceptive effects of xylazine, detomidine and romifidine on experimental pain in horses. *Veterinary Anaesthesia and Analgesia* 30, 183-190.

Nguyen D, Abdul-Rasool I, Ward D et al. (1992) Ventilatory effects of dexmedetomidine, atropine, and isoflurane in dogs. *Anesthesiology* 76, 573-579.

Nelson BB, Lordan EE, Hassel DM. (2013) Risk factors associated with gastrointestinal dysfunction in horses undergoing elective procedures under general anaesthesia. *Equine Veterinary Journal Supplement* 45, 8-14.

Neudeck et al. (2018a) Pharmacodynamics and plasma concentrations of dexmedetomidine with and without vatinoxan as a constant-rate infusion in horses anaesthetized with isoflurane. *In World Congress of Veterinary Anesthesiology Proceedings 2018 Venice*.

Neudeck S, Kästner SB, Wittenberg-Voges L et al. (2018b) Comparison of desflurane and propofol at equipotent doses in combination with a constant rate infusion of dexmedetomidine on global and peripheral perfusion and oxygenation in horses. *American Journal of Veterinary Research* 79, 487-495.

Niimura Del Barrio, M C, Bennett RC, Hughes JML. (2017) Effect of detomidine or romifidine constant rate infusion on plasma lactate concentration and inhalant requirements during isoflurane anaesthesia in horses. *Veterinary Anaesthesia and Analgesia* 44, 473-482.

Nilsfors L, Kvart C. (1986) Preliminary report on the cardiorespiratory effects of the antagonist to detomidine, MPV-1248. *Acta Veterinaria Scandinavica* 82, 121-129.

Nishida T, Nishimura M, Kagawa K et al. (2002) The effects of dexmedetomidine on the ventilatory response to hypercapnia in rabbits. *Intensive Care Medicine* 28, 969-975.

Nyman G, Funkquist B, Kvart C et al. (1990) Atelectasis causes gas exchange impairment in the anaesthetised horse. *Equine Veterinary Journal* 22, 317-324.

Nyman G, Marntell S, Edner A et al. (2009) Effect of sedation with detomidine and butorphanol on pulmonary gas exchange in the horse. *Acta Veterinaria Scandinavica* 51, 22.

Olsen E, Pedersen TLS, Robinson R et al. (2016) Accuracy and precision of oscillometric blood pressure in standing conscious horses. *Journal of Veterinary Emergency and Critical Care* 26, 85-92.

Pagel PS, Proctor LT, Devcic A et al. (1998) A novel alpha2-adrenoceptor antagonist attenuates the early, but preserves the late cardiovascular effects of intravenous dexmedetomidine in conscious dogs. *Journal of Cardiothoracic and Vascular Anesthesia* 12, 429-434.

Pakkanen SA, Raekallio MR, Mykkänen AK et al. (2015) Detomidine and the combination of detomidine and MK-467, a peripheral alpha-2 adrenoceptor antagonist, as premedication in horses anaesthetized with isoflurane. *Veterinary Anaesthesia and Analgesia* 42, 527-536.

Pakkanen SAE, de Vries A, Raekallio MR et al. (2018) Changes in energy metabolism, and levels of stress-related hormones and electrolytes in horses after intravenous administration of romifidine and the peripheral α-2 adrenoceptor antagonist vatinoxan. *Acta Veterinaria Scandinavica* 60, 27.

Pardridge WM. (2012) Drug transport across the blood–brain barrier. *Journal of Cerebral Blood Flow & Metabolism* 32, 1959-1972.

Perotta JH, Canola PA, Lopes MC et al. (2014) Hyoscine-N-butylbromide premedication on cardiovascular variables of horses sedated with medetomidine. *Veterinary Anaesthesia and Analgesia* 41, 357-364.

Pertovaara A, Haapalinna A, Sirviö J et al. (2005) Pharmacological properties, central nervous system effects, and potential therapeutic applications of atipamezole, a selective α_2-adrenoceptor antagonist. *CNS Drug Reviews* 11, 273-288.

Philipp M, Brede M, Hein L. (2002) Physiological significance of α_2-adrenergic receptor subtype diversity: one receptor is not enough. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 283, R287-295.

Pimenta EL, Teixeira Neto FJ, Sa PA et al. (2011) Comparative study between atropine and hyoscine-N-butylbromide for reversal of detomidine induced bradycardia in horses. *Equine Veterinary Journal* 43, 332-340.

Potter JJ, MacFarlane PD, Love EJ et al. (2016) Preliminary investigation comparing a detomidine continuous rate infusion combined with either morphine or buprenorphine for standing sedation in horses. *Veterinary Anaesthesia and Analgesia* 43, 189-194.

Pypendop BH, Verstegen JP. (1998) Hemodynamic effects of medetomidine in the dog: A dose titration study. *Veterinary Surgery* 27, 612-622.

Pypendop BH, Escobar A, Siao KT et al. (2013) Effect of dexmedetomidine on its clearance: a pharmacokinetic model. *Journal of Veterinary Pharmacology and Therapeutics* 36, 89-91.

Pypendop BH, Honkavaara J, Ilkiw JE. (2017) Pharmacokinetics of dexmedetomidine, MK-467 and their combination following intramuscular administration in male cats. *Veterinary Anaesthesia and Analgesia* 44, 823-831.

Raekallio M, Leino A, Vainio O et al. (1992) Sympatho-adrenal activity and the clinical sedative effect of detomidine in horses. *Equine Veterinary Journal Supplement* 11, 66-68.

Raekallio M, Vainio O, Karjalainen J. (1990) The influence of atipamezole on the cardiovascular effects of detomidine in horses. *Veterinary Anaesthesia and Analgesia* 17, 50-53.

Raekallio MR, Honkavaara JM, Vainio OM. (2010) The effects of L-659,066, a peripheral α_2-adrenoceptor antagonist, and verapamil on the cardiovascular influences of

dexmedetomidine in conscious sheep. *Journal of Veterinary Pharmacology and Therapeutics* 33, 434-438.

Raisis AL, Young LE, Blissitt KJ et al. (2000) Effect of a 30-minute infusion of dobutamine hydrochloride on hind limb blood flow and hemodynamics in halothane-anesthetized horses. *American Journal of Veterinary Research* 61, 1282-1288.

Ramseyer B, Schmucker N, Schatzmann U et al. (1998) Antagonism of detomidine sedation with atipamezole in horses. *Veterinary Anaesthesia and Analgesia* 25, 47-51.

Rang HP, Ritter JM, Flower RJ et al. (2016a) Noradrenergic transmission In: *Rang & Dale's Pharmacology* 8th ed. Elsevier Churchill Livingstone London (UK) Chapter 14, 177-196.

Rang HP, Ritter JM, Flower RJ et al. (2016b) Cholinergic transmission In: *Rang & Dale's Pharmacology* 8th ed. Elsevier Churchill Livingstone London (UK) Chapter 13, 155-176.

Ranheim B, Risberg AI, Spadavecchia C et al. (2015) The pharmacokinetics of dexmedetomidine administered as a constant rate infusion in horses. *Journal of Veterinary Pharmacology and Therapeutics* 38, 93-96.

Reitemeyer H, Klein HJ, Deegen E. (1986) The effect of sedatives on lung function in horses. *Acta Veterinaria Scandinavica Supplementum* 82, 111-120.

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

Restitutti F, Laitinen MR, Raekallio MR et al. (2013) Effect of MK-467 on organ blood flow parameters detected by contrast-enhanced ultrasound in dogs treated with dexmedetomidine. *Veterinary Anaesthesia and Analgesia* 40, 48-56.

Restitutti F, Kaartinen MJ, Raekallio MR et al. (2017) Plasma concentration and cardiovascular effects of intramuscular medetomidine combined with three doses of the peripheral alpha$_2$-antagonist MK-467 in dogs. *Veterinary Anaesthesia and Analgesia* 44, 417-426.

Rezende ML, Grimsrud KN, Stanley SD et al. (2015) Pharmacokinetics and pharmacodynamics of intravenous dexmedetomidine in the horse. *Journal of Veterinary Pharmacology and Therapeutics* 38, 15-23.

Ringer SK, Kalchofner K, Boller J et al. (2007) A clinical comparison of two anaesthetic protocols using lidocaine or medetomidine in horses. *Veterinary Anaesthesia and Analgesia* 34, 257-268.

Ringer SK, Schwarzwald CC, Portier KG et al. (2013) Effects on cardiopulmonary function and oxygen delivery of doses of romifidine and xylazine followed by constant rate infusions in standing horses. *The Veterinary Journal* 195, 228-234.

Risberg A, Spadavecchia C, Ranheim B et al. (2014) Antinociceptive effects of three escalating dexmedetomidine and lignocaine constant rate infusions in conscious horses. *The Veterinary Journal* 202, 489-497.

Risberg AI, Ranheim B, Krontveit RI et al. (2016) The cardiovascular status of isoflurane-anaesthetized horses with and without dexmedetomidine constant rate infusion evaluated at equivalent depths of anaesthesia. *Veterinary Anaesthesia and Analgesia* 43, 412-423.

Roekaerts PM, Prinzen FW, De Lange S. (1996) Beneficial effects of dexmedetomidine on ischaemic myocardium of anaesthetized dogs. *British Journal of Anaesthesia* 77, 427-429.

Roelvink ME, Goossens L, Kalsbeek HC et al. (1991) Analgesic and spasmolytic effects of dipyrone, hyoscine-N-butylbromide and a combination of the two in ponies. *Veterinary Record* 129, 378-380.

Roger T, Ruckebusch Y. (1987) Colonic α_2-adrenoceptor-mediated responses in the pony. *Journal of Veterinary Pharmacology and Therapeutics* 10, 310-318.

Rohrbach H, Korpivaara T, Schatzmann U et al. (2009) Comparison of the effects of the alpha-2 agonists detomidine, romifidine and xylazine on nociceptive withdrawal reflex and temporal summation in horses. *Veterinary Anaesthesia and Analgesia* 36, 384-395.

Rolfe NG, Kerr CL, McDonell WN. (2012) Cardiopulmonary and sedative effects of the peripheral α_2-adrenoceptor antagonist MK 0467 administered intravenously or intramuscularly concurrently with medetomidine in dogs. *American Journal of Veterinary Research* 73, 587-594.

Rosenbaum DM, Rasmussen SG, Kobilka BK. (2009) The structure and function of G-protein-coupled receptors. *Nature* 459, 356-363.

Rossetti RB, Cortopassi SRG, Intelizano T et al. (2008) Comparison of ketamine and S (+)-ketamine, with romifidine and diazepam, for total intravenous anesthesia in horses. *Veterinary Anaesthesia and Analgesia* 35, 30-37.

Ruffolo RR, Hieble JP. (1994) α-Adrenoceptors. *Pharmacology & Therapeutics* 61, 1-64.

Sabbe MB, Penning JP, Ozaki GT et al. (1994) Spinal and systemic action of the α_2 receptor agonist dexmedetomidine in dogs. Antinociception and carbon dioxide response. *Anesthesiology* 80, 1057-1072.

Sacks M, Ringer SK, Bischofberger AS et al. (2017) Clinical comparison of dexmedetomidine and medetomidine for isoflurane balanced anaesthesia in horses. *Veterinary Anaesthesia and Analgesia* 44, 1128-1138.

Salla KM, Tuns CI, Bennett RC et al. (2017) Cardiovascular effects of premedication with medetomidine alone and in combination with MK-467 or glycopyrrolate in dogs subsequently anesthetized with isoflurane. *American Journal of Veterinary Research* 78, 1245-1254.

Sallinen J, Link RE, Haapalinna A et al. (1997) Genetic alteration of α_{2C}-adrenoceptor expression in mice: influence on locomotor, hypothermic, and neurochemical effects of dexmedetomidine, a subtype-nonselective α_2-adrenoceptor agonist. *Molecular Pharmacology* 51, 36-46.

Salonen JS. (1986) Pharmacokinetics of detomidine. *Acta Veterinaria Scandinavica Supplementum* 82, 59-66.

Salonen JS, Vähä-Vahe T, Vainio O et al. (1989) Single-dose pharmacokinetics of detomidine in the horse and cow. *Journal of Veterinary Pharmacology and Therapeutics* 12, 65-72.

Salonen JS, Eloranta M. (1990) Biotransformation of medetomidine in the rat. *Xenobiotica* 20, 471-480.

Samuels ER, Szabadi E. (2008) Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part II: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans. *Current Neuropharmacology* 6, 254-285.

Sasaki N, Yoshihara T, Hara S. (2000) Difference in the motile reactivity of jejunum, cecum, and right ventral colon to xylazine and medetomidine in conscious horses. *Journal of Equine Science* 11, 63-68.

Saternos HC, Almarghalani DA, Gibson HM et al. (2018) Distribution and function of the muscarinic receptor subtypes in the cardiovascular system. *Physiological Genomics* 50, 1-9.

Schafers RF, Elliott HL, Howie CA et al. (1992) A preliminary, clinical pharmacological assessment of L-659,066, a novel α_2-adrenoceptor antagonist. *British Journal of Clinical Pharmacology* 34, 521-526.

Schauvliege S, Marcilla MG, Verryken K et al. (2011) Effects of a constant rate infusion of detomidine on cardiovascular function, isoflurane requirements and recovery quality in horses. *Veterinary Anaesthesia and Analgesia* 38, 544-554.

Schauvliege S, Gasthuys F. (2013) Drugs for cardiovascular support in anesthetized horses. *Veterinary Clinics of North America: Equine Practice* 29, 19-49.

Scheibner J, Trendelenburg AU, Hein L et al. (2002) α_2-adrenoceptors in the enteric nervous system: a study in α_{2A}-adrenoceptor-deficient mice. *British Journal of Pharmacology* 135, 697-704.

Scheinin M, MacDonald E. (1989) An introduction to the pharmacology of α_2-adrenoceptors in the central nervous system. *Acta Veterinaria Scandinavica Supplementum* 85, 11-19.

Scheinin M, Lomasney JW, Hayden-Hixson DM et al. (1994) Distribution of α_2-adrenergic receptor subtype gene expression in rat brain. *Molecular Brain Research* 21, 133-149.

Schwartz DD, Clark TP. (1998) Affinity of detomidine, medetomidine and xylazine for alpha-2 adrenergic receptor subtypes. *Journal of Veterinary Pharmacology and Therapeutics* 21, 107-111.

Schwinn DA. (1993) Adrenoceptors as models for G protein-coupled receptors: structure, function and regulation. *British Journal of Anaesthesia* 71, 77-85.

Senior JM, Pinchbeck GL, Allister R et al. (2006) Post anaesthetic colic in horses: a preventable complication? *Equine Veterinary Journal* 38, 479-484.

Singh S, McDonell W, Young S et al. (1997) The effect of glycopyrrolate on heart rate and intestinal motility in conscious horses. *Veterinary Anaesthesia and Analgesia* 24, 14-19.

Solano AM, Valverde A, Desrochers A et al. (2009) Behavioural and cardiorespiratory effects of a constant rate infusion of medetomidine and morphine for sedation during standing laparoscopy in horses. *Equine Veterinary Journal* 41, 153-159.

Steffey EP, Dunlop CI, Farver TB et al. (1987) Cardiovascular and respiratory measurements in awake and isoflurane-anesthetized horses. *American Journal of Veterinary Research* 48, 7-12.

Stegmann GF, Littlejohn A. (1987) The effect of lateral and dorsal recumbency on cardiopulmonary function in the anaesthetised horse. *The Journal of the South African Veterinary Association* 58, 21-27.

Sundra TM, Harrison JL, Lester GD et al. (2012) The influence of spasmolytic agents on heart rate variability and gastrointestinal motility in normal horses. *Research in Veterinary Science* 93, 1426-1433.

Sutton D, Preston T, Christley RM et al. (2002) The effects of xylazine, detomidine, acepromazine and butorphanol on equine solid phase gastric emptying rate. *Equine Veterinary Journal* 34, 486-492.

Szemeredi K, Stull R, Kopin IJ et al. (1989) Effects of a peripherally acting α_2-adrenoceptor antagonist (L-659,066) on hemodynamics and plasma levels of catechols in conscious rats. *European Journal of Pharmacology* 170, 53-59.

Tapio H, Argüelles D, Gracia-Calvo LA et al. (2017) Modified technique for common carotid artery transposition in standing horses. *Veterinary Surgery* 46, 52-58.

Teixeira Neto FJ, McDonell WN, Black WD et al. (2004) Effects of glycopyrrolate on cardiorespiratory function in horses anesthetized with halothane and xylazine. *American Journal of Veterinary Research* 65, 456-463.

Tobin G, Giglio D, Lundgren O. (2009) Muscarinic receptor subtypes in the alimentary tract. *Journal of Physiology and Pharmacology* 60, 3-21.

Vainionpää MH, Raekallio MR, Pakkanen SA et al. (2013) Plasma drug concentrations and clinical effects of a peripheral alpha-2-adrenoceptor antagonist, MK-467, in horses sedated with detomidine. *Veterinary Anaesthesia and Analgesia* 40, 257-264.

Valverde A, Rickey E, Sinclair M et al. (2010) Comparison of cardiovascular function and quality of recovery in isoflurane-anaesthetised horses administered a constant rate infusion of lidocaine or lidocaine and medetomidine during elective surgery. *Equine Veterinary Journal* 42, 192-199.

Van Zwieten PA, Timmermans P. (1984) Central and peripheral α-adrenoceptors. Pharmacological aspects and clinical potential. *Advances in Drug Research* 13, 209-254.

Vincent JL, De Backer D. (2004) Oxygen transport-the oxygen delivery controversy. *Intensive Care Medicine* 30, 1990-1996.

Virtanen R. (1986) Pharmacology of detomidine and other α_2-adrenoceptor agonists in the brain. *Acta Veterinaria Scandinavica Supplementum* 82, 35-46.

Virtanen R, Savola JM, Saano V et al. (1988) Characterization of the selectivity, specificity and potency of medetomidine as an α_2-adrenoceptor agonist. *European Journal of Pharmacology* 150, 9-14.

Wagner AE, Muir WW, Hinchcliff KW. (1991) Cardiovascular effects of xylazine and detomidine in horses. *American Journal of Veterinary Research* 52, 651-657.

Wehrwein EA, Orer HS, Barman SM. (2016) Overview of the anatomy, physiology, and pharmacology of the autonomic nervous system. *Comprehensive Physiology* 37, 125.

Wilffert B, Timmermans PB, Van Zwieten PA. (1982) Extrasynaptic location of alpha-2 and noninnervated beta-2 adrenoceptors in the vascular system of the pithed normotensive rat. *Journal of Pharmacology and Experimental Therapeutics* 221, 762-768.

Wilson DV, Bohart GV, Evans AT et al. (2002) Retrospective analysis of detomidine infusion for standing chemical restraint in 51 horses. *Veterinary Anaesthesia and Analgesia* 29, 54-57.

Wittenberg-Voges L, Kastner SB, Raekallio M et al. (2018) Effect of dexmedetomidine and xylazine followed by MK-467 on gastrointestinal microperfusion in anaesthetized horses. *Veterinary Anaesthesia and Analgesia* 45, 165-174.

Wooldridge AA, Eades SC, Hosgood GL et al. (2002) In vitro effects of oxytocin, acepromazine, detomidine, xylazine, butorphanol, terbutaline, isoproterenol, and dantrolene on smooth and skeletal muscles of the equine esophagus. *American Journal of Veterinary Research* 63, 1732-1737.

Yaksh TL. (1985) Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. *Pharmacology Biochemistry & Behavior* 22, 845-858.

Yamashita K, Yonezawa K, Izumisawa Y et al. (1996) Antagonistic effects of atipamezole on medetomidine-induced sedation in horses. *Journal of Veterinary Medical Science* 58, 1049-1052.

Yamashita K, Tsubakishita S, Futaok S et al. (2000) Cardiovascular effects of medetomidine, detomidine and xylazine in horses. *Journal of Veterinary Medical Science* 62, 1025-1032.

Young SS, Taylor PM. (1993) Factors influencing the outcome of equine anaesthesia: a review of 1,314 cases. *Equine Veterinary Journal* 25, 147-151.

Zhang L, Keef KD, Bradley ME et al. (1992) Action of α_{2A}-adrenergic receptors in circular smooth muscle of canine proximal colon. *American Journal of Physiology* 262, 517.

Zullian C, Menozzi A, Pozzoli C et al. (2011) Effects of alpha2-adrenergic drugs on small intestinal motility in the horse: an in vitro study. *The Veterinary Journal* 187, 342-346.