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NEW APPLICATIONS OF ALPHA₂-AGONISTS IN VETERINARY MEDICINE

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Sommario

SOMMARIO	2
ABSTRACT.....	4
RIASSUNTO	6
ALPHA₂-ADRENOCEPTOR AGONISTS IN VETERINARY MEDICINE	8
INTRODUCTION.....	9
MECHANISM OF ACTION OF ALPHA ₂ -ADRENOCEPTOR AGONISTS	11
SEDATIVE EFFECTS	18
ANALGESIC EFFECTS.....	27
CARDIOVASCULAR EFFECTS.....	32
RESPIRATORY EFFECTS.....	37
OTHER EFFECTS	39
ALPHA ₂ -ANTAGONISTS	44
NEW APPLICATIONS OF ALPHA₂-AGONISTS.....	46
IN VETERINARY MEDICINE.....	46
ALPHA ₂ -AGONISTS FOR SEMEN COLLECTION	47
DEXMEDETOMIDINE INFUSION DURING SURGERY IN TWO DOGS UNDERGOING ADRENALECTOMY FOR A SUSPICION OF A PHEOCHROMOCYTOMA	69
ALPHA ₂ -AGONISTS FOR THE PERIOPERATIVE MANAGEMENT OF ANESTHESIA IN NON-HUMAN PRIMATES UNDERGOING CRANIOTOMY	82
DISCUSSION AND CONCLUSION	90
REFERENCES	93

Abstract

Alpha₂-agonists are a class of drugs widely used in veterinary anaesthesia; moreover by means of their action on adrenoceptors that are widespread distributed in several tissues, they can be beneficial for different clinical applications. The aim of this work was to describe new applications of alpha₂-agonists in veterinary medicine.

In cats, high dose medetomidine is administered to perform semen collection by urethral catheterization. We have investigated the haemodynamic effects of high dose medetomidine (0.13 mg kg⁻¹) administered to healthy male cats. Haemodynamic evaluations were performed before and after medetomidine administration and consisted of: clinical examination, blood pressure evaluation and transthoracic echocardiography. Significant hemodynamic alterations were observed, even if they were similar to that provided by lower dosages. The cats recovered without clinical alterations.

Despite their cardiovascular side effects, low doses of alpha₂-agonists can be beneficial for the maintenance of a good cardiovascular stability for specific conditions.

In humans, dexmedetomidine helps in maintaining a good hemodynamic stability if administered for pheochromocytoma ablation. We have described the administration of dexmedetomidine for the anesthetic management of two dogs with a suspicion of pheochromocytoma undergoing adrenalectomy. Dogs received dexmedetomidine intramuscularly (0.001 mg kg⁻¹) and dexmedetomidine and remifentanyl were administered (0.0005 mg kg⁻¹h⁻¹ and 0.0003 mg kg⁻¹min⁻¹, respectively) throughout the surgery. In this study dexmedetomidine infusion together with remifentanyl provided satisfactory intraoperative anesthetic and hemodynamic control in two dogs with a suspicion of pheochromocytoma.

In patients undergoing craniotomy, dexmedetomidine, increasing the cerebral vascular resistance, prevents alteration of the cerebral blood flow. We have described the administration of dexmedetomidine in five *Macaca fascicularis* undergoing craniotomy for physiologic research. The

Macaca were sedated with ketamine (8 mg kg^{-1}) and dexmedetomidine (0.02 mg kg^{-1}) intramuscularly. Dexmedetomidine was administered by infusion ($0.012 \text{ mg kg}^{-1}\text{h}^{-1}$) throughout the procedure and provided adequate analgesia and a stable hemodynamic control in healthy Macaca.

Riassunto

I farmaci alfa₂-agonisti sono largamente utilizzati in anestesia veterinaria; inoltre, grazie alla loro azione sui recettori alpha-adrenergici, distribuiti in diversi tessuti, sono utilizzati per diverse applicazioni cliniche. L'obiettivo del presente studio è stato quello di descrivere nuove applicazioni degli alfa₂-agonisti in medicina veterinaria.

Nel gatto, la medetomidina somministrata ad alte dosi consente la raccolta del seme mediante cateterismo uretrale. Abbiamo valutato gli effetti emodinamici della medetomidina somministrata al dosaggio di 0.13 mg kg⁻¹ in gatti sani. Le valutazioni emodinamiche sono state eseguite prima e dopo la somministrazione di medetomidina mediante visita clinica, misurazione della pressione sistemica ed ecocardiografia transtoracica. Dallo studio sono state evidenziate alterazioni emodinamiche significative, ma simili a quelle riportate dopo somministrazione di dosi più basse.

I farmaci alfa₂-agonisti, nonostante le alterazioni cardiovascolari che inducono, se somministrati a basse dosi, possono contribuire al mantenimento di una buona stabilità emodinamica in condizioni cliniche specifiche.

Nell'uomo, la somministrazione di dexmedetomidina in pazienti sottoposti a rimozione di un feocromocitoma contribuisce a mantenere parametri emodinamici intraoperatori stabili. Abbiamo descritto la somministrazione perioperatoria di dexmedetomidina in due cani sottoposti a surrenalectomia per un sospetto di feocromocitoma. Entrambi hanno ricevuto dexmedetomidina intramuscolo (0.001 mg kg⁻¹) e dexmedetomidina e remifentanil sono stati somministrati in infusione (0.0005 mg kg⁻¹h⁻¹ e 0.0003 mg kg⁻¹min⁻¹, rispettivamente) per tutta la chirurgia. Il protocollo utilizzata ha permesso di mantenere un piano anestesilogico e condizioni emodinamiche stabili in due cani con sospetto di feocromocitoma.

Nei pazienti sottoposti a neurochirurgia, la dexmedetomidina previene alterazioni significative del flusso cerebrale. Abbiamo descritto la somministrazione di dexmedetomidina in esemplari di

Macaca fascicularis sottoposti a craniotomia. I macachi sono stati sedati con ketamina e dexmedetomidina. La dexmedetomidina è stata somministrata in infusione continua ($0.012 \text{ mg kg}^{-1}\text{h}^{-1}$) per tutta la procedura e ha permesso di mantenere un'analgesia adeguata e parametri emodinamici stabili in macachi sani.

Alpha₂-adrenoceptor agonists in veterinary medicine

Introduction

Alpha₂-adrenoceptor agonists are a class of drugs widely used in veterinary anesthesia to obtain sedation, analgesia and muscle relaxation. Their application has been described in small companion animals (Sinclair et al. 2003; Lemke 2004), in large animals (Daunt and Steffey, 2002; Valverde 2010; Gozalo-Marcilla et al. 2015) and in laboratory animals (Lee et al. 2010; Lugo-roman et al. 2010) among others.

The alpha₂-adrenoceptor drugs most commonly used in clinical practice are xylazine, detomidine, romifidine, medetomidine and dexmedetomidine.

Xylazine was first synthesized in Germany in 1962 as an antihypertensive drug but its sedative properties were soon discovered in animals, becoming the first alpha₂-agonist used in veterinary anesthesia. It was first used in ruminants, then in horses and cats (since 1970s), and finally in dogs (Green and Thurmon 1988; Paddleford and Harvey 1999).

The administration of alpha₂-agonists also produces side effects which must be taken into consideration. A decrease in heart rate and arrhythmias, and a biphasic pressure response are the most problematic side effects described after alpha₂-agonist administration at therapeutic doses. In addition, several endocrine effects have been recognized: decreased insulin release, glycogenolytic effects and decreased antidiuretic hormone release.

Since alpha₂ adrenoceptors are involved in the regulation of several physiological mechanisms, their administration may provide multiple effects other than those sedative and analgesic.

Among other applications, studies in veterinary medicine have reported their administration in male animals of several species for semen collection. In cats, high doses of medetomidine (0.13 mg kg⁻¹) are needed to obtain semen release into the urethra. Since the effects of high dose medetomidine in cats have never been described, the aim of this study was to investigate the hemodynamic effects induced by medetomidine administered intramuscularly in healthy cats at a dose of 0.13 mg kg⁻¹.

In addition, dexmedetomidine administration has been reported to improve cardiovascular stability in human patients with pheochromocytoma and in those undergoing pheochromocytoma ablation. In dogs, adrenalectomy is the treatment of choice for patients with pheochromocytomas; however, mortality is high due to catecholamine release during mass manipulation. Alpha₂-agonists have been described to reduce the catecholamine release; therefore, one of the following studies described the use of dexmedetomidine infusion as an adjunct to a balanced anesthetic protocol in two dogs undergoing pheochromocytoma ablation.

Finally, dexmedetomidine has been described as a potential supplemental anesthetic drug in human patients undergoing intracranial surgery. To the best of our knowledge, there are no studies evaluating the use of dexmedetomidine as an adjunct for the anesthetic management of veterinary patients undergoing craniotomy. Therefore, we aimed to describe the use of dexmedetomidine administered by continuous rate infusion (CRI) for the anesthetic management of *Cynomolgus monkeies* used as animal models and undergoing craniotomy for the purpose of physiologic research.

Mechanism of action of α_2 -adrenoceptor agonists

α_2 -agonists exert their action by means of agonism on the α_2 -adrenoceptors. In addition, the majority of them, except for xylazine, have an imidazoline ring and can be combined with imidazoline receptors (Khan et al. 1999; Clarke et al. 2014).

Alpha- adrenoceptors

Alpha-adrenoceptors are differentiated on the basis of their sensitivity to agonists and antagonists (Table 1). α_2 -adrenoceptors are located presynaptically and postsynaptically in both central and peripheral sites while α_1 -adrenoceptors are only postsynaptic. Norepinephrine (NA) is the natural ligand for both α_1 - and α_2 -adrenoceptors; when NA is released from a sympathetic neuron, it acts on both receptors inducing sympathetic stimulation. When activated, presynaptic α_2 -adrenoceptors exert a negative feedback and prevent the additional release of NA, decreasing the sympathetic outflow from the central nervous system (CNS) (Figure 1). Postsynaptic α_1 and α_2 -adrenoceptors usually exert a stimulating effect.

α_2 -adrenoceptors are transmembrane receptors formed by a long chain of amino acids with hydrophilic and hydrophobic areas which cross the cell membrane seven times (Khan et al. 1999). α_2 -adrenoceptors belong to the family of guanine nucleotide-binding proteins (G-proteins)-coupled receptors. G-proteins are formed by alpha, beta and gamma subunits, and have been identified on the intracellular side of the cell membrane. The alpha subunit at rest binds to guanosine diphosphate (GDP). When the receptor is activated by its agonist, it changes its structure binding with the G-protein which reduces its affinity for GDP and, in the presence of magnesium, it becomes guanosine triphosphate (GTP). The alpha subunit leaves the G-protein, reaching the effector and, concurrently, the agonist leaves the receptor (Figure 2). The GTPase, which is now activated, hydrolyzes GTP to GDP with the release of an inorganic phosphate, and the receptor becomes inactive (Taylor 1990; Khan et al. 1999).

Alpha₂-adrenoceptors are involved in more than one effector mechanism. Their activation through the inhibitory G-protein (Gi-protein) inhibits the adenylyl cyclase, resulting in the decreased formation of 3',5'-cyclic adenosine monophosphate (cAMP), the second messenger of many biological mechanisms (Khan et al. 1999). The activation of the stimulatory G-protein (Gs- protein) causes hyperpolarization of the neuronal cells. However, other studies have suggested that the effect resulting from the activation of alpha₂-adrenoceptors depends only on the agonist concentration; a low alpha₂-agonist concentration results in the inhibition of cAMP while high agonist concentration induces an increase in cAMP (Eason et al. 1992; Gyires et al. 2009).

An alternative mechanism involves voltage-gated calcium ion channels coupled to a G-protein; this theory states that the decrease in calcium ion conductance results in the inhibition of the neurotransmitter release (Khan et al. 1999).

The presynaptic alpha₂-adrenoceptors involved in the inhibition of the NA release are mainly located in sympathetic nerve endings and in non-adrenergic neurons in the CNS. Postsynaptic alpha₂-adrenoceptors have been identified in several tissues, such as the liver, pancreas, platelets, kidney, adipose tissue and the eye, where they are involved in several physiologic functions (Khan et al. 1999).

Four alpha₂-adrenoceptor subtypes have been identified on the basis of pharmacological and molecular studies: alpha_{2A}, alpha_{2B}, and alpha_{2C}. The fourth type, alpha_{2D}, identified in the rat brain is the homologue of human alpha_{2A}.

In the CNS, the alpha_{2A}-subtype has been identified in the brain and mainly in the locus ceruleus, the alpha_{2B}-subtype has been found only in the thalamus and the alpha_{2C}-subtype is widely distributed but is mainly present in the basal ganglia (MacDonald and Scheinin 1995). Radioligand binding assays and real time polymerase chain reaction (PCR) have confirmed the presence of the alpha_{2A}-adrenoceptor subtype in the dog brainstem (Schwartz et al. 1999).

The most commonly used alpha₂-agonists are characterized by a different selectivity towards the alpha₁- and the alpha₂ adreceptors (Table 2). This difference in selectivity towards alpha-

adrenoceptors explains the differences in the sedative and physiologic effects obtained with their administration. Schwartz and Clark (1998) evaluated the affinity of xylazine, detomidine and medetomidine for the different adrenoceptor subtypes. They found that detomidine and medetomidine have a higher affinity for all receptors when compared to xylazine.

Receptor type	Specific drug	Action
Alpha ₁ -adrenoceptors	Epinephrine	Agonist
	Norepinephrine	
	Phenylephrine	
	Methoxamine	
	Prazosin	Antagonist
Alpha ₂ -adrenoceptors	Epinephrine	Agonist
	Norepinephrine	
	Clonidine	
	Yohimbine	Antagonist

Table 1-Classification of alpha-adrenoceptors based on their sensitivity for agonists and antagonists (Clarke et al. 2014)

Drug	Selectivity alpha ₂ : alpha ₁ receptors
Xylazine	160:1
Detomidine	260:1
Romifidine	340:1
Medetomidine	1620:1
Dexmedetomidine	1620:1

Table 2- alpha₂: alpha₁ receptor selectivity (Khan et al. 1999; Muir 2009)

Imidazole receptors

On the cell membranes, imidazoline receptors are always associated with α_2 -adrenoceptors with the unique exception of chromaffin cells (Farsang and Kaposci 1999).

Three subtypes of imidazoline receptors have been identified:

- I1 receptors have been identified on the cell membranes in the ventrolateral medulla, in the cortex and in other regions of the brain. They have also been described outside the brain on chromaffin cells, on platelets and in the glomus caroticum (Farsang and Kaposci 1999). I1 receptors are involved in blood pressure regulations; their activation induces hypotension.

- I2 receptors have been localized on the outer side of the mitochondrial membrane. I2 receptors have been identified mainly in the brain (in the cortex and in the nucleus striatum) but also in several viscera: the pancreas, prostate, urethra, placenta, kidney, colon, endothelial cells, glomus caroticum, adrenal medulla, platelets and adipocytes (Farsang and Kaposci 1999; Khan et al. 1999).

- Not I1-Not I2 receptors have been identified on the axon terminal of the sympathetic neurons. In rabbits, these receptors have been described in the aorta, pulmonary artery, heart and iris; in rats they have been identified in the lungs and in the kidneys (Farsang and Kaposci 1999). The endogenous ligands of imidazoline receptors are: clonidine displacing substance (CDS), immunoreactive CDS and agmatine, involved in the regulation of several biological effects (Farsang and Kaposci 1999).

The mechanism of action of imidazoline receptors has not yet been clarified. Authors have hypothesized that the I1 receptor is coupled with a G-protein and activates choline phospholipid hydrolysis with the subsequent formation of products, such as diacylglyceride, arachidonic acid, prostaglandin and eicosanoids (Ernsberger et al. 1995; Ernsberger 1999; Farsang and Kaposci 1999). Moreover, the activation of this receptor inhibits the Na^+/H^+ exchange and is involved in the activation of enzymes for catecholamines (Ernsberger 1999). The I2 receptor does not seem to be linked to a G-protein but, instead, to be coupled with a potassium channel (Okumara et al. 1992).

The imidazoline receptors are not involved in sedation; however, due to their widespread distribution, they regulate several mechanisms. They control catecholamine synthesis in the adrenal medulla, they modulate blood pressure and intraocular pressure, and they are involved in the modulation of glucose metabolism and body temperature (Ernsberger et al. 1995; Farsang and Kapocsi 1999). Central imidazole receptors have been demonstrated to be involved in the hypotensive and anti-arrhythmic effects mediated by clonidine and dexmedetomidine, respectively (Kamibayashi et al. 1995).

Sedative effects

The α_2 -adrenoceptor agonists are widely used in veterinary medicine to obtain sedation and anxiolysis. α_2 -agonist binding with α_{2A} -adrenoceptors located in the *locus coeruleus* prevents additional NA release with subsequent sedation (Sinclair 2003).

A study in rats has demonstrated that dexmedetomidine administration into the locus coeruleus induces sedation in a dose-dependent manner which is mediated by α_2 -adrenoceptors. In fact, this hypnotic effect was reversed by the systemic administration of an α_2 -adrenoceptor antagonists, such as atipamezole, which crossed the blood brain barrier (Correa-Sales et al. 1992). Instead, the administration of α_1 - or beta- adrenoceptor antagonists did not reverse the sedation (Doze et al. 1989).

The α_2 -agonists most commonly used for sedation in dogs, cats and horses are xylazine, detomidine, romifidine, medetomidine and dexmedetomidine. They can be used alone or in combination with an opioid to improve the quality of sedation.

α_2 -agonists are also commonly administered by constant rate infusion (CRI) as part of balanced anesthetic protocols in several species (Bettschart-Wolfensberger et al. 2001; Carter et al. 2010; Pypendop et al. 2011; Pascoe 2015). Their intraoperative administration has been proven to reduce the mean alveolar concentration (MAC) of inhalant anesthetics, or the dose of propofol or alfaxalone required for the maintenance of a stable anesthetic plane (Ewing et al. 1993; Neges et al. 2003; Ringer et al. 2007). In equine anesthesia, the adjuncts of α_2 -agonists by CRI to a balanced anesthetic protocol assure a better quality of recovery as well as a stable anesthetic plane (Ringer et al. 2007; Gozalo-Marcilla et al. 2015). In horses, the sedative effects observed after α_2 -agonist administration are decreased awareness, drop of the head, ptosis of the eyelids and of the lower lip, and ataxia (Valverde 2010).

Xylazine

Xylazine has been used in clinical practice since the 1960s. It does not have any imidazole ring and, therefore, it does not act on imidazole receptors (Clarke et al. 2014).

Xylazine provides a rapid onset of sedation after intramuscular (IM) and intravenous (IV) administration, while subcutaneous administration (SC) is less reliable and provides only poor sedation (Clarke et al. 2014; Rankin 2015).

In horses, xylazine is used to obtain sedation for standing procedures or as a premedicant drug before the induction of general anesthesia (Green and Thurmon 1988; Ringer et al. 2013; Rankin 2015). Suggested doses for this species are 0.5-1.0 mg kg⁻¹ and 1.0-2.0 mg kg⁻¹ for the IV and IM routes, respectively. Xylazine administered to horses at 1.1 mg kg⁻¹ provides deep sedation and analgesia within 5-10 minutes, lasting 30-60 minutes and with an elimination half-life of approximately 50 minutes (Garcia-Villar et al. 1981; Jochle et al. 1991; Rankin 2015). The combination of xylazine, ketamine and guaifenesin (also known as triple drip) has been described for the maintenance of intravenous general anesthesia in horses. This protocol has been recommended only for procedures lasting less than 1 hour (Davidson 2008).

Cattle are particularly sensitive to xylazine, especially the Brahman breed; lower initial doses should be used in these cattle for sedation (Green and Thurmon 1988). In this species, deaths have been reported from hypoxemia after xylazine administration (Clarke et al. 2014). In cattle, xylazine 0.2 mg kg⁻¹ reached its peak plasma concentration 15 minutes after IM administration and had an elimination half-life of 30 minutes (Garcia-Villar et al. 1981).

In dogs and cats, xylazine has been used for short-term sedation to perform diagnostic procedures or as a premedicant agent prior to general anesthesia (Green and Thurmon 1988; Paddlerford and Harvey 1999). The recommended dose of xylazine in dogs and cats ranges from 0.25 to 0.5 mg kg⁻¹ IV and 0.5 to 1.0 mg kg⁻¹ IM. In dogs, xylazine 1.4 mg kg⁻¹ administered IM reached its peak plasma concentration after 15 minutes and had an elimination half-life of 30 minutes (Garcia-Villar et al. 1981). Xylazine has recently been described in association with ketamine in dogs to provide smooth, safe and effective total intravenous anesthesia (TIVA) for minor procedures (Ibrahim 2017). The

combination of ketamine (2 mg kg^{-1} IV for induction followed by an infusion of $10 \text{ mg kg}^{-1} \text{ h}^{-1}$) in combination with xylazine (1 mg kg^{-1} IV followed by $1 \text{ mg kg}^{-1} \text{ h}^{-1}$) provided a stable anesthetic plane for 60 minutes characterized by smooth induction and recovery, and good muscle relaxation without any paddling or tonic-clonic movement. The animals were able to stand 36 minutes after the disconnection of 60 minutes of anesthesia infusion (Ibrahim 2017).

Detomidine

Detomidine is more commonly used in horses and cattles than in small animals, and its potency has been shown to be similar among several species (Clarke et al. 2014). Detomidine is more potent than xylazine, and effective sedation can be achieved with lower doses; however, it has a wide therapeutic index (Rankin 2015).

In horses, detomidine can be used as a premedicant drug before general anesthesia but is more commonly used as a sedative agent for standing procedures. Conversely when either detomidine ($0.005 \text{ mg kg}^{-1} \text{ h}^{-1}$) or saline solution were administered by CRI in isoflurane-anesthetized horses, the authors did not find any differences in isoflurane requirement and quality of recovery between the two groups (Schauvliege et al. 2011). For standing procedures, even after high doses of detomidine, horses maintain the standing position with lower ataxia as compared with sedation provided by other alpha2-agonists (Clarke et al. 2014). In horses, detomidine given IV at 0.04 mg kg^{-1} has a median half-life of 26 minutes (Hubbell et al. 2009; Rankin 2015). If the same dose is given to horses soon after exercise, the median half-life and the median volume of distribution increase significantly. This difference is mainly due to a different distribution of the cardiac output observed after exercise (Hubbell et al. 2009). These results might explain why higher dosages are necessary to obtain effective sedation in horses after exercise.

For more invasive standing procedures, such as laparoscopy or dental procedures, detomidine administered by CRI alone or in association with opioids has been reported to provide effective sedation and analgesia (Cruz et al. 2004; Virgin et al. 2010; Potter et al. 2016). Potter and colleagues

have described a protocol for standing dental procedures using acepromazine (0.02 mg kg^{-1}) and detomidine 0.01 mg kg^{-1} followed by detomidine CRI $0.0006 \text{ mg kg}^{-1} \text{ min}^{-1}$ in combination with either buprenorphine (0.01 mg kg^{-1}) or morphine (0.1 mg kg^{-1}). Both protocols provided effective sedation with the horses receiving buprenorphine having a higher sedation score and a higher incidence of side effects in the postoperative period (box walking, abdominal pain and shivering) (Potter et al. 2016). The concurrent administration of methadone (0.2 mg kg^{-1}) as an adjunct to low doses of detomidine ($0.0025 \text{ mg kg}^{-1}$) did not provide more sedation than that observed after the administration of detomidine alone (0.005 mg kg^{-1}) (Gozalo-Marcilla et al. 2017). A higher degree of sedation was observed when methadone was administered with higher doses of detomidine (0.01 mg kg^{-1}), with the peak of the sedative effect observed 15 minutes after administration.

In ruminants, detomidine provided a more potent sedation when compared to xylazine but has been used to a lesser extent. In this species, it provides sedation similar to that provided in horses (Riebold 2015).

In both calves and horses, the sublingual administration of an oro-mucosal gel formulation of detomidine has been described (Kaukinen et al. 2011; Hokkanen et al. 2014). In horses, detomidine gel administered at a dose of 0.04 mg kg^{-1} induced sedation within 40 min. In 54% of the horses receiving oral detomidine, the sedation was scored moderate to heavy while in 41% of the horses, only mild sedation was obtained (Gardner et al. 2010). In calves, detomidine gel has a bioavailability of 34%, and a sublingual dose of 0.08 mg kg^{-1} has provided effective sedation similar to that obtained with the IV administration of detomidine 0.01 mg kg^{-1} (Hokkanen et al. 2014).

Romifidine

Romifidine is widely used in horses, and it is labelled for use in this species in several countries. However, its use has also been described in small animals (Muir and Gadawski 2002; Selmi et al. 2002).

In horses, romifidine ($0.08\text{--}0.12\text{ mg kg}^{-1}$) produces profound sedation for standing procedures with less ataxia as compared with other α_2 -agonists (Clarke et al. 2014; Rankin 2015). A study has reported that only one out of ten horses stumbled or fell forward 5 minutes after the administration of romifidine 0.08 or 0.12 mg kg^{-1} (Freeman and England 2000). In this species, romifidine is commonly used as a premedicant agent but it is also administered by CRI during general anesthesia.

Wojtasiak-Wypart and colleagues (2012) have reported that, when romifidine was administered IV to horses at 0.08 mg kg^{-1} , peak sedation occurred within 15 minutes, and the sedative effect lasted up to 2 hours, with an elimination half-life of 138 minutes.

More recently we have described the partitioning of romifidine in red blood cells after IV administration at a dose of 0.1 mg kg^{-1} (Romagnoli et al. 2017). The Authors found that, after IV administration, romifidine concentration in the red blood cells was two times higher than that in the plasma, and that the ratio between the two concentrations was constant over time (Figure 3). Romifidine has been detected in red blood cells up to 180 minutes after IV injection. This effect is explained by the lipophilicity of this drug; it can pass the membrane of the red blood cells binding to the membrane or to the intracellular molecules; as soon as the plasma concentration decreases, the drug is slowly released from the cells. The plasma concentration of romifidine has been found to be higher than 10 mg ml^{-1} in 75% of the animals included in the study from 30 to 180 minutes after IV administration. The Authors have reported that romifidine (0.1 mg kg^{-1}) provided good sedation in all horses from 2 minutes after administration, lasting up to 105 minutes; however, half of the horses included were still sedated 120 minutes after injection and one horse up to 150 minutes. The Authors have hypothesized that romifidine partitioning in the red blood cells could explain the long-lasting sedative effect observed in horses after its administration (Romagnoli et al. 2017). In the same study, the mean clearance was $22.16 \pm 6.67\text{ ml min}^{-1}\text{ kg}^{-1}$, and the mean half-life of distribution and elimination was 119.28 (range 51.6–248.9) minutes.

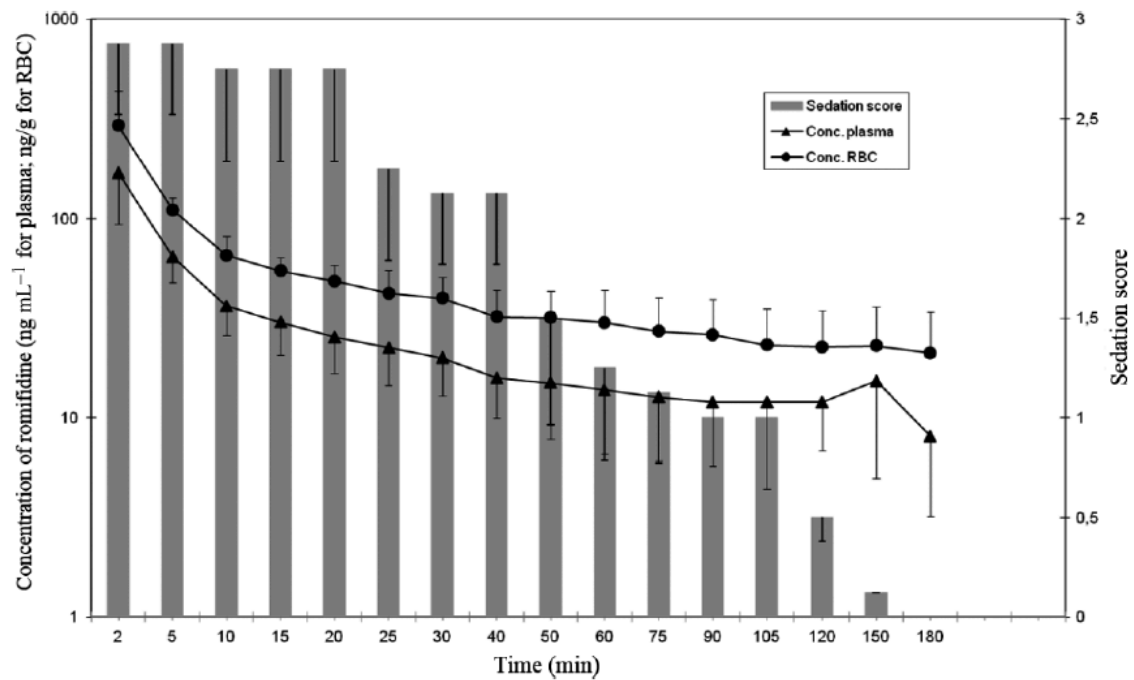


Figure 3- Plasma and red blood cells concentration of romifidine and sedation score versus time observed in 8 horses after intravenous administration of romifidine 0.1 mg kg⁻¹ (from Romagnoli N, Al-Qudah KM, Armorini S, Lambertini C, Zaghini A, Spadari A, Roncada P (2017) *Pharmacokinetic profile and partitioning in red blood cells of romifidine after single intravenous administration in the horse. Vet Med Sci* 3, 187-197)

There is some discrepancy among studies concerning romifidine sparing effects on inhalant anesthetics. In isoflurane-anesthetized horses, romifidine, administered by CRI at 0.04 mg kg⁻¹ h⁻¹ (following a bolus of romifidine 0.08 mg kg⁻¹ IV), has been shown to provide an isoflurane sparing effect of 15% as compared to horses premedicated with xylazine (1 mg kg⁻¹) and receiving saline solution by CRI during surgery (Niimura Del Barrio et al. 2017). In the same study, romifidine infusion did not significantly alter the duration or the quality of recovery as compared with the control group. However, a previous study, in which an equal dose of romifidine was administered to isoflurane-anesthetized horses, had failed to find any sparing effects (Devisscher et al. 2010). The differences among the studies may have been due to the fact that different surgical procedures were performed on the horses involved.

In dogs, romifidine ($0.01\text{--}0.08\text{ mg kg}^{-1}$) has been reported to produce effective sedation and a sparing effect on the dose of propofol necessary for the induction of general anesthesia (Lemke 1999, England et al. 1996; Gomez-Villamandos et al. 2005)

Medetomidine

Medetomidine is a racemic mixture, widely used in both small and large animals (Bryant et al. 1991; Sinclair 2003; Ringer et al. 2007).

In horses, medetomidine (0.007 mg kg^{-1}) produced more ataxia with respect to other α_2 -agonists up to recumbency (Bryant et al. 1991; Bettschart-Wolfensberger et al. 1999). For this reason, and due to its short duration of action, it is not commonly used for standing procedures but more often for premedication followed by CRI during general anesthesia (Ringer et al. 2007; Clarke et al. 2014). Its intraoperative administration, in horses anesthetized with isoflurane, helps in maintaining a stable anesthetic plane, reducing the inhalant anesthetic requirement as well as having a good cardiovascular stability (Bettschart-Wolfensberger et al. 1999, 2001). Of the other α_2 -agonists, medetomidine has the shortest half-life and the most rapid clearance rate. In horses, when medetomidine was administered IV as a single bolus (0.007 mg kg^{-1}), its mean elimination half-life was 51.3 min and its total body clearance was $4.0\text{ ml kg}^{-1}\text{ h}^{-1}$ (Bettschart-Wolfensberger et al. 1999). In the same species, medetomidine (0.01 mg kg^{-1}) reaches peak plasma concentration 6.4 minutes after IV administration, with an elimination half-life of 29 minutes (Grimsrud et al. 2012).

In small animals, medetomidine has been used as a single bolus for sedation or as CRI during general anesthesia. In dogs, doses of 0.03 mg kg^{-1} have been reported to provide similar sedation to xylazine 2.2 mg kg^{-1} (Cullen 1996; Vainio et al. 1989). In the same species, medetomidine 0.04 mg kg^{-1} administered IV provided sedation in 15-20 minutes corresponding to a plasma concentration of 18.5 ng ml^{-1} (Kuusela et al. 2000). The administration of a low dose CRI of medetomidine ($0.1\text{ mg kg}^{-1}\text{ h}^{-1}$) as part of a balanced anesthetic protocol contributed to the maintenance of a stable anesthetic plane and provided effective analgesia in dogs undergoing ovariohysterectomy (Rioja et al. 2013).

The more commonly used dosages suggested in clinical practice for obtaining effective sedation in dogs range from 0.02 to 0.15 mg kg⁻¹; the duration of the sedative effect is dose dependent (Stenberg et al. 1987).

In cats, medetomidine is commonly combined with ketamine or opioids to achieve effective sedation (Slingsby et al. 2015). The combination of medetomidine (0.03 mg kg⁻¹) with buprenorphine (0.02 mg kg⁻¹) has been reported to significantly reduce the amount of inhalant anesthetic required for maintenance of general anesthesia as compared with medetomidine alone (Grint et al. 2009). The addition of medetomidine (0.02 mg kg⁻¹) to butorphanol (0.2 mg kg⁻¹) has been reported to be more effective than the addition of acepromazine (0.2 mg kg⁻¹) in reducing the dose of alfaxalone necessary for the induction and maintenance of general anesthesia (Schwarz et al. 2014).

Dexmedetomidine

Dexmedetomidine is the D-isomer of medetomidine and has a selectivity $\alpha_2:\alpha_1$ ratio of 1300:1 and an α_2 :imidazoline selectivity ratio of 32:1 (Khan et al. 1999). Dexmedetomidine, administered at half the dose of medetomidine, has been reported to provide similar effects (Kuusela et al. 2000; Bettschart-Wolfensberger et al. 2005).

In horses, dexmedetomidine has a rapid distribution and a short duration of action. For this reason, in this species, it is administered by CRI as part of a balanced anesthetic protocol in order to provide a constant level of sedation and to improve the recovery quality (Bettschart-Wolfensberger et al. 2005; Marcilla et al. 2012; Marly-Voquer et al. 2016). In equine species, its half-life of elimination, after a single bolus (0.0035 mg kg⁻¹), is 28.96 ± 7.61 minutes (Bettschart-Wolfensberger et al. 2005).

In dogs, dexmedetomidine (0.02 mg kg⁻¹) provides similar sedation to that obtained with the administration of medetomidine (0.04 mg kg⁻¹) but with a longer lasting effect (Kuusela et al. 2000). The concurrent administration of levomedetomidine (0.01-0.02 mg kg⁻¹) did not provide any adjunctive effect to the sedation obtained with dexmedetomidine alone. Conversely, higher doses of

levomedetomidine (0.08 mg kg^{-1}) reduced the sedative and analgesic effects of dexmedetomidine when administered together (Kuusela et al. 2000, 2001). In dogs sedated with dexmedetomidine (0.01 and 0.02 mg kg^{-1}), the peak sedative effect was obtained 10 to 20 minutes after IV administration, corresponding to a plasma concentration of 5.5 ± 3.0 and $14.0 \pm 4.5 \text{ ng ml}^{-1}$, respectively (Kuusela et al. 2000). Dexmedetomidine, administered as a CRI ($0.0015 \text{ mg kg}^{-1} \text{ h}^{-1}$ and $0.0045 \text{ mg kg}^{-1} \text{ h}^{-1}$) in sevoflurane-anesthetized dogs produced a dose-dependent decrease in the MAC of the inhalant anesthetic (Hector et al. 2017).

In cats, dexmedetomidine (0.01 mg kg^{-1}) has been used alone to obtain effective sedation, even if the addition of opioids or ketamine provided more adequate sedation without increasing the incidence of adverse cardiovascular effects. In particular, its combination with butorphanol (0.2 mg kg^{-1}) decreased the incidence of vomiting (Bhalla et al. 2017). When dexmedetomidine, at doses of 0.02 - 0.04 mg kg^{-1} , was combined with alfaxalone (5 mg kg^{-1}), general anesthesia was obtained (Selmi et al. 2003; Rodrigo-Mocholí et al. 2016). Dexmedetomidine administered to cats as a premedicant drug before general anesthesia significantly reduced the dosage of the induction agent and the MAC of isoflurane in a plasma concentration related manner (from 0.006 to 11.46 ng ml^{-1}) (Escobar et al. 2012; McSweeney et al. 2012). Dexmedetomidine administered at a dose of 0.025 mg kg^{-1} reached its peak plasmatic concentration (10.2 ng ml^{-1}) 17.8 minutes (range 2.6- 44.9) after IM administration (Pypendop et al. 2017).

The oral administration (PO) of dexmedetomidine has been described in humans and small animal practice. In dogs dexmedetomidine is well absorbed systemically through the oral mucous membrane and this method of administration was effective for the sedation of fractious and fearful dogs (Cohen and Bennett 2015). In cats, the oral or the IM administration of dexmedetomidine has similar systemic bioavailability with comparable sedative and antinociceptive effects (Slingsby et al. 2009).

Analgesic effects

The analgesic effect of α_2 -agonists is the result of their action at the central and spinal levels. At the spinal level, they bind to α_{2A} , α_{2B} and α_{2C} receptors in the dorsal horn of the spinal cord. The activation of noradrenergic receptors inhibits the NA and the substance P release from A δ and C fibers, thereby inhibiting the central transmission of the afferent nociceptive stimuli (Valverde 2010). In both the brain and the spinal cord, the α_2 -adrenoceptors interact with the opioid receptors and share the same signal transduction system, the G protein. Opioid inhibition of nociceptive transmission at the spinal level might be mediated by the activation of the α_2 -adrenoceptors. Among opioid receptors, the δ -subtype has been found to be the main subtype involved in interaction with the adrenergic receptors (Omote et al. 1991). Studies support the interaction between α_2 -adrenergic receptors and opioid receptors. For example, Ossipov and colleagues (1989) have demonstrated that systemic yohimbine attenuates the nociceptive effect of intrathecally-administered morphine and clonidine.

Studies have suggested that the analgesic effect of α_2 -agonists is dose dependent but shorter lasting when compared to the sedative effect (Kramer et al. 1996). The analgesia provided by these drugs, even when locally administered, is reversed by the administration of the specific antagonist (Sabbe et al. 1994; Sinclair 2003).

Systemic administration

The effect of the systemic administration of α_2 -agonists has been evaluated on both somatic and visceral nociception. Their application has been described in clinical trials and in experimental models applying thermal and mechanical thresholds, or duodenal and colorectal distension for somatic and visceral evaluation respectively. The results change with species and the drugs used, and also depend on the stimulus applied.

In horses, xylazine has been used to provide sedation and analgesia since it was first introduced in veterinary practice (Daunt and Steffey 2002). The analgesic effect of xylazine has been compared to opioids in a model of visceral analgesia in which colic was experimentally induced by inflating a balloon in the cecum (Muir and Robertson 1985). In that study xylazine (1.1 mg kg^{-1}) provided a more effective and longer lasting analgesia as compared with that provided by butorphanol (0.2 mg kg^{-1}), meperidine (1.0 mg kg^{-1}) and penthazocine (0.99 mg kg^{-1}). In another study regarding the same species, the combination of xylazine with opioids (morphine, butorphanol or nalbuphine) did not provide any adjunctive analgesia for dental dolorimetry as compared to xylazine administered alone (Brunson and Majors 1987). When xylazine was combined with butorphanol it potentiated its analgesic effect up to 30 to 60 minute after administration (Brunson and Majors 1987).

In a clinical study on horses with signs of colic, detomidine (0.020 and 0.040 mg kg^{-1}) has been used to relieve abdominal pain and was more effective in decreasing signs of pain than xylazine (0.5 mg kg^{-1}), but also compared to butorphanol (0.1 mg kg^{-1}) and flunixin meglumine (1.0 mg kg^{-1}) (Jochle et al. 1989). In the same species, an experimental study has been reported that detomidine (0.010 and 0.020 mg kg^{-1}) significantly increased the colorectal and duodenal distension threshold without affecting the thermal threshold (Elfenbein et al. 2009).

Alpha₂-agonists have also been used to relieve pain in patients undergoing standing surgeries. In mares, undergoing a standing flank laparotomy for ovariectomy and oophorectomy, detomidine (0.02 mg kg^{-1}) provided better analgesia than the combination of xylazine and morphine (Jochle et al. 1991).

The efficacy of alpha₂-agonists in managing surgical pain has also been described in small animals (Sinclair 2003; Beckman 2013). In dogs, medetomidine alone (0.01 , 0.03 , 0.09 , 0.18 mg kg^{-1}) has been described to provide similar analgesic effect to xylazine (2.2 mg kg^{-1}) in response to the application of superficial pain stimuli (Vainio et al. 1989). Another study has demonstrated that, in this species, medetomidine (0.04 and 0.08 mg kg^{-1}) alone provided analgesia for only a minimally invasive procedure (Kramer et al. 1996)

Dexmedetomidine has been compared to morphine for the management of postoperative analgesia in dogs undergoing abdominal or thoracic surgery, or spinal neurosurgery (Valtolina et al. 2009). In that study, dogs receiving dexmedetomidine (0.001 mg kg^{-1} followed by an infusion of $0.001 \text{ mg kg}^{-1} \text{ h}^{-1}$) or morphine (0.1 mg kg^{-1} followed by $0.1 \text{ mg kg}^{-1} \text{ h}^{-1}$) had similar pain scores during the first 12 hours after surgery even if more dogs receiving dexmedetomidine required rescue analgesia (11/20) as compared with those treated with morphine (8/20) (Valtolina et al. 2009).

In dogs, the combination of dexmedetomidine with morphine or methadone has been demonstrated to be more effective than dexmedetomidine alone (0.01 mg kg^{-1}) in suppressing the withdrawal reflex induced by the application of toe pinches (Cardoso et al. 2014).

Combined α_2 -agonists and opioids also potentiate their analgesic effect in cats. In a study on cats, dexmedetomidine (0.020 mg kg^{-1}) combined with buprenorphine (0.010 mg kg^{-1}) significantly increased the thermal nociceptive threshold from 0.25 to 1.25 hours after administration (Slingsby et al. 2015). This effect was greater than the same doses of buprenorphine or dexmedetomidine administered alone.

Local administration

The local administration of α_2 -agonists has been described for epidural analgesia, local infiltration, peripheral nerve block and intraarticular infiltration (Campoy and Read 2013).

α_2 -agonists, by means of their action on α_{2A} -adrenoceptors, enhance the action of local anesthetic drugs (Yoshitomi et al. 2008). In an experimental study on guinea pigs, dexmedetomidine has been proven to increase the degree and the duration of the analgesic effects of lidocaine in a dose-dependent manner when injected intracutaneously. In the same study the improved anesthetic effect was reversed by yohimbine (α_{2A} , α_{2B} , and α_{2C} adrenoceptor antagonists) but not by prozosin (α_1 , α_{2B} , and α_{2C} adrenoceptor antagonists) (Yoshitomi et al. 2008).

In an experimental study on rats, dexmedetomidine (0.0005, 0.002, 0.006 and 0.02 mg kg⁻¹) was added to ropivacaine 0.75% to achieve a sciatic nerve block, and the sensory blockade to the application of a heat stimulus was evaluated (Brummet et al. 2009). The addition of dexmedetomidine increased the duration of the sensory block in a dose-dependent manner; however, when dexmedetomidine was used alone, no significant motor or sensory block was obtained (Brummet et al. 2008). At histopathological evaluation, a mild perineural inflammation was recorded 24 hours after the experiment; however, no signs of perineural inflammation were found 14 days later. Moreover, the highest dose of dexmedetomidine (0.20 mg kg⁻¹) did not provoke any histological nerve damage (Brummet et al. 2009).

In cats, the combination of dexmedetomidine (0.001 mg kg⁻¹) with bupivacaine (0.46 mg kg⁻¹) in achieving a sciatic and femoral nerve block did not decrease the response to toe pinching and did not increase the paw withdrawal threshold as compared to bupivacaine administered alone (Evangelista et al. 2017). Furthermore, in a previous study on dogs, dexmedetomidine (0.0001 mg kg⁻¹) combined with ropivacaine (0.74 mg kg⁻¹) to achieve a sciatic and femoral nerve block did not increase the duration of the sensory block (Trein et al. 2017). These results differ from human studies which used dexmedetomidine in combination with local anesthetic drugs to perform local anesthetic techniques. Marhofer and colleagues (2013) have reported that in human volunteers, dexmedetomidine (0.02 mg kg⁻¹) administered with ropivacaine (0.75%) to achieve an ulnar nerve block, increased the duration of the sensory block as compared with ropivacaine alone when the pinprick test was applied.

Epidurally administered α_2 -agonists exert their analgesic effect by means of their action at the spinal level; however, systemic absorption cannot be excluded (Sabbe et al. 1994; Steagall et al. 2017). In dogs, epidurally or intrathecally administered dexmedetomidine provided comparable antinociceptive effects to systemically administered dexmedetomidine but without any signs of sedation (Sabbe et al. 1994). A study has compared the effects of the epidural administration of

lidocaine 2% alone or in combination with α_2 -agonists (xylazine 0.25 mg kg⁻¹, romifidine 0.01 mg kg⁻¹, detomidine 0.03 mg kg⁻¹, dexmedetomidine 0.002 mg kg⁻¹ or clonidine 0.005 mg kg⁻¹) in dogs undergoing ovariohysterectomy (Pohl et al. 2012). Among the other α_2 -agonists, xylazine has been shown to determine a longer lasting analgesic effect lasting up to 4 hours (Pohl et al. 2012). In the same study, 3 out of the forty-two dogs involved which received detomidine, 4 which received xylazine and 5 which received clonidine required inhalation anesthesia in addition to the epidural; all bitches receiving epidural dexmedetomidine required isoflurane anesthesia.

In dogs undergoing orthopedic surgery, epidural medetomidine (0.015 mg kg⁻¹) has provided analgesia comparable to epidurally administered oxymorphone, but a higher incidence of cardiovascular side effects has been recorded. The epidural administration of medetomidine (0.005 mg kg⁻¹) combined with morphine (0.1 mg kg⁻¹) did not provide any benefit as compared to the use of medetomidine alone (Vesal et al. 1996; Pacharinsak et al. 2003).

In horses, epidural detomidine has frequently been used to provide effective analgesia for standing procedures. Epidurally administered detomidine induced analgesia extending from the coccyx up to T15 and, as compared to xylazine, induced a higher degree of cardiovascular side effects and a more frequent change in hind limb position (Skarda and Muir 1996). When continuous IV or epidural detomidine infusions have been compared in mares undergoing standing ovariectomy, no significant differences in hormonal responses have been detected. However, mares which had not been received the local analgesia had a higher visual analogue scale (VAS) score when more painful stimuli were applied (Virgin et al. 2010).

The intraarticular administration of α_2 -agonists has also been described (Soto et al. 2014). In dogs undergoing stifle joint surgery, intraarticular administered dexmedetomidine (0.0025 mg kg⁻¹) has provided postoperative analgesia comparable to intraarticular administered morphine (0.1 mg kg⁻¹), lasting up to 6 hours (range 2-10 hours). Dogs treated with the combination of the two drugs did not require additional analgesia for up to 10 hours (range 4-14 hours).

Cardiovascular effects

Alpha₂-adrenoceptor agonists, by means of their action on alpha₂-adrenoceptors, can significantly impair the cardiovascular system. Bradycardia, arrhythmia, decreased cardiac output (CO) and increased systemic vascular resistance (SVR) are the most commonly reported alterations after alpha₂-adrenoceptor agonist administration in dogs, cats and horses among others (England and Clarke 1996; Pypendop and Verstegen 1998; Ko et al. 2001; Lamont et al. 2001; Carter et al. 2010).

In an experimental study, dexmedetomidine (0.0005 mg kg⁻¹min⁻¹) has been demonstrated to prevent arrhythmia induced by epinephrine in halothane-anesthetized dogs (Kamibahyashi et al. 1995). In that study, the authors hypothesized that this anti-arrhythmogenic effect was mediated by the action of dexmedetomidine on central imidazole receptors. In fact, the administration of two imidazole- alpha₂-antagonists inhibited or reversed the dexmedetomidine action while the effect of non-imidazole alpha₂-antagonists was not significant.

Heart rate

In several species, the administration of alpha₂-agonists has usually been associated with a decrease in heart rate (HR) with respect to baseline values (Golden et al. 1998; Pypendop and Verstegen 1998; Ko et al. 2001; Lamont et al. 2001; Ilbäck and Stålhandske 2003; Carter et al. 2010).

In small animals, medetomidine has been reported to induce a significant decrease in HR, and some authors have reported that this effect is not dose dependent; at low dosages, the decrease in HR is less pronounced and, at higher dosages, it is longer lasting (Pypendop and Verstegen 1998). Medetomidine administered by CRI in dogs (0.001-0.003 mg kg⁻¹ min⁻¹), has also been reported to decrease the HR (Carter et al. 2010). A decrease in HR has also been reported in cats after medetomidine and romifidine administration, with medetomidine decreasing the HR up to 68% from baseline 15 minutes after IM administration (Lamont et al. 2001; Muir and Gadawski 2002).

Clonidine, xylazine, detomidine, medetomidine and romifidine are reported to produce profound bradycardia and atrioventricular (AV) blocks when administered to horses (Wagner et al. 1991; England and Clarke 1996). In equine species, xylazine (1.1 mg kg^{-1}) has been found to induce a less profound and a shorter-lasting alteration on HR than that of equipotent doses of detomidine (0.02 mg kg^{-1}) and romifidine (0.08 mg kg^{-1}) (Wagner et al. 1991; England et al. 1992).

There are two main mechanisms involved in the reduction of the HR observed after the administration of α_2 -adrenoceptor agonists. The interaction of these drugs with the central α_2 -adrenoceptors reduces the sympathetic outflow, thereby reducing the HR. In addition, the interaction with peripheral α_2 -adrenoceptors results in increased systemic vascular resistance (SVR) and subsequent reflex bradycardia (Sinclair et al. 2003). The direct effects of α_2 -agonists on intrinsic myocardial contractility have been excluded in a study carried out regarding the isolated ventricular myocardium of ferrets (Housman 1990).

Preemptive atropine administration has been hypothesized to reduce the incidence of bradycardia mediated by α_2 -agonists. However, the increase in HR induced by anticholinergic drugs in the presence of a peripheral vasoconstriction due to the α_2 -agonists would increase the risk of arrhythmia (Ko et al. 2001).

Arterial blood pressure

α_2 -agonist administration is usually characterized by a biphasic pressure response; soon after the drug administration, an initial increase in blood pressure is followed by a hypotensive phase (Pypendop and Verstegen 1998; Ilbäck and Stålhandske 2003).

This biphasic pattern has mainly been observed after the administration of higher doses of medetomidine (Pypendop and Verstegen 1998). When low doses of medetomidine are administered, central effects predominate, and hypotension is more frequently observed. Higher doses of medetomidine exert a more pronounced action on peripheral adrenoceptors with subsequent

vasoconstriction and a significant increase in arterial blood pressure (Pypendop and Verstegen 1998; Ko et al. 2001). However, as the peripheral action ceases, the central effect persists, and the decreased CO contributes to the normalization or the decrease of the systemic arterial pressure (SAP) (Carter et al. 2010). The hypertensive effect is seen especially after the IV administration of medetomidine; in fact, after IM administration, the peak blood concentration is delayed.

Higher doses of α_2 -agonists can cause a significant increase in blood pressure which can be detrimental in patients affected by heart or cardiovascular disease. In a study in dogs, the administration of a high dose of medetomidine (0.02 mg kg^{-1}) induced an increase in the SAP up to 200 mmHg even if of short duration (Pypendop and Verstegen 1998). Medetomidine 0.01 mg kg^{-1} given intravenously has been reported to cause sudden cardiac arrest due to the rupture of an aortic aneurysm in a dog affected by *Spirocerca lupi* (Joubert et al. 2005)

The biphasic response has been described in horses after the administration of clonidine, xylazine, detomidine, medetomidine or romifidine (England and Clarke 1996). Detomidine 0.02 mg kg^{-1} IV induced initial hypertension soon after its administration which lasted 15 minutes. This initial increase was then followed by a significant decrease in the mean arterial pressure (MAP) with respect to the baseline values 1 to 2 hours after administration (Wagner et al. 1991). On the other hand, Wagner and colleagues (1991) have observed that xylazine 1.1 mg kg^{-1} IV and 2.2 mg kg^{-1} IM administered in healthy horses induced a decrease in MAP 5 minutes after administration which became statistically significant 15 minutes later and lasted up to 120 minutes.

Studies in mice have demonstrated that the central hypotensive response after α_2 -agonists administration is mediated by the α_{2A} -adrenoceptor subtype while the increase in systemic blood pressure is mediated by α_{2B} -adrenoceptors (Link et al. 1996; MacMillan et al. 1996). In fact, in α_{2B} -deficient mice, the hypertensive response has not been observed after dexmedetomidine administration. On the contrary, the disruption of the α_{2C} -subtype did not produce any hemodynamic changes with respect to the control animals (Link et al. 1996). In mice with a mutation

of the α_{2A} -subtype, the hypotensive response was lost after the administration of an α_2 -agonist (MacMillan et al. 1996).

Data concerning the effect of α_2 -adrenoceptor agonists on pulmonary circulation are controversial, and vary among drugs and species. In the pulmonary vessels, there is a low density of α_2 -agonists when compared to the systemic circulation, and neuronal regulation plays a minor role in their regulation (Pypendop and Verstegen 1998; Lamont et al. 2001). In small animals, medetomidine did not affect the pulmonary vascular resistance index (PVRI) or the mean pulmonary arterial pressure (Pypendop and Verstegen 1998; Lamont et al. 2001). In horses, detomidine and xylazine induced a significant increase in the PVRI (Wagner et al. 1991). Moreover, while xylazine did not affect the mean pulmonary arterial pressure, low and high dose detomidine induced a significant decrease in the mean pulmonary arterial pressure.

Cardiac output

The administration of α_2 -adrenoceptor agonists has been reported to induce a transient decrease in both cardiac output (CO) and the cardiac index (CI) (England and Clarke 1996; Pypendop and Verstegen 1998; Carter et al. 2010).

The CO is determined by complex and coupled variables, but primarily depends on HR and stroke volume (SV). The SV, that is the volume of blood pumped by the heart in each cycle, is influenced by preload, afterload, contractility and lusitropic properties (Muir 2015). A study on the isolated ventricular papillary muscles of ferrets has demonstrated that dexmedetomidine has no intrinsic contractile effects on the myocardium (Housman 1990). There is a discrepancy among authors concerning the effect of α_2 -adrenoceptor agonists on SV and the stroke index (SI) in small and large animals (Pypendop and Verstegen 1998; Yamashita et al. 2000; Carter et al. 2010). However, it is likely that the commonly observed decrease in CO is mainly due to bradycardia or to decreased contractility related to reduced sympathetic tone.

Stroke Volume decreased in automatically blocked dogs after medetomidine administration (de Moraes and Muir 1995). In healthy dogs, medetomidine 0.001 mg kg^{-1} induced a significant decrease in CO and the CI without significant alterations in the SI. A lesser decrease in the CI index has been reported after the administration of low dose medetomidine (Pypendop and Verstegen 1998). In cats, medetomidine 0.02 mg kg^{-1} induced a significant decrease in the CI up to 37% of baseline 15 minutes after administration; the value remained at 50% of baseline 30 minutes later (Lamont et al. 2001). In horses treated with xylazine or detomidine, the CO was significantly reduced by both drugs administered at different dosages; however, detomidine administration ($0.02 \text{ mg kg}^{-1} \text{ IV}$) was associated with a lower CO and CI lasting up to 60 minutes (Wagner et al. 1991). In this latter study, the decrease in CO lasted longer than the reduction of the HR. However, in the absence of significant alterations in SV or the SI the authors recognized the decrease in HR as the main cause of the decreased CO.

In another study on horses treated with different dosages of medetomidine, detomidine or xylazine, the SV decreased from baseline, even if the variation was not statistically significant (Yamashita et al. 2000).

Respiratory effects

The administration of α_2 -agonists alone has been reported to decrease in f_R due to CNS depression (Sinclair 2003). Respiratory depression has not always been associated with a significant alteration in blood gas tension (Lamont et al. 2001). In healthy dogs, low dose medetomidine (0.005 and 0.01 mg kg⁻¹) reduced the sensitivity of the response to increased fractional concentration of inspired carbon dioxide (FiCO₂). This affected the f_R , the tidal volume and the minute volume (Lerche and Muir 2004). In horses, xylazine (0.01 mg kg⁻¹) induced a significant decrease in the partial arterial pressure of oxygen (PaO₂) without altering the partial pressure of carbon dioxide in arterial blood (PaCO₂) (Lavoie et al. 1992a).

In horses, the sedation provided by α_2 -agonists is commonly associated with head and neck dropping. The alteration in head carriage causes an increase in hydrostatic pressure which results in nasal mucosa congestion which contributes to increased airway resistance. In addition, these drugs provide a relaxation of the nostrils and head dropping which changes the conformation of the thorax and of the lungs. Taken together, these factors contribute to alterations in ventilator mechanics (Lavoie et al. 1992b).

In dogs, peripheral cyanosis has commonly been observed after medetomidine administration, also in healthy dogs. Sinclair (2003) has suggested that low blood flow through the peripheral capillary bed and high oxygen extraction were the main mechanisms inducing a cyanotic mucous appearance in α_2 -treated animals.

Ruminants, and especially sheep among others, are more sensitive to the respiratory depression induced by these drugs, even at low doses. Studies have described profound hypoxemia in sheep after the administration of xylazine, romifidine, detomidine, medetomidine or dexmedetomidine, despite their α receptor selectivity, and without a significant increase in PaCO₂ (Celly et al. 1997; Kästner et al. 2007). On the basis of their results, some authors have hypothesized that the hypoxia was not due to hypoventilation, to changes in body position or to the degree of sedation. Celly and colleagues

(1997) concluded that the increase in shunt fraction and the alteration of pulmonary mechanics, with a subsequent increase in $P(A-a)O_2$ and a decrease in transpulmonary pressure recorded after the administration of all the α_2 -agonists tested, were the main causes of hypoxemia (Celly et al. 1997). The hydrostatic stress, characterized by increased capillary pressure, and protein and erythrocyte extravasation was considered to be the main cause of the pulmonary edema and capillary congestion observed in dexmedetomidine-treated sheeps (Kastner et al. 2007)

The degree of respiratory depression induced by α_2 -agonists increases when they are combined with other drugs, such as opioids. With these combinations, oxygenation of the patient is strongly recommended, especially in critically ill animals.

Other effects

Glycemic effect

Alpha₂-agonists have been reported to increase serum glucose concentration and to decrease insulin levels in several species (Feldberg and Symonds 1980; Ambrisko and Hikasa 2002; Restitutti et al. 2012). This hyperglycemic effect induced by alpha₂-agonists has been reversed with the administration of alpha₂-antagonists (atipamezole, yohimbine and MK-467) (Maroto et al. 1992; Ambrisko and Hikasa 2002; Restitutti et al. 2012).

Xylazine has been described to induce hyperglycemia in dogs and cats (Feldberg and Symonds 1980; Ambrisko and Hikasa 2002). In dogs xylazine (1-8 mg kg⁻¹) increased blood glucose dose dependently from two hours after IM administration and to a greater extent than medetomidine administered at equipotent sedative doses. In healthy dogs, the effect of medetomidine on glucose concentration depends on the dose administered (Ambrisko and Hikasa 2002). Medetomidine, administered at 0.01 mg kg⁻¹, did not significantly alter blood glucose levels (Maroto et al. 1992; Burton et al. 1997) while higher dosages (0.02-0.08 mg kg⁻¹) induced a significant increase in blood glucose (Maroto et al. 1992).

In another study, low dose medetomidine (0.005 mg kg⁻¹ IM) also induced a significant increase in plasma glucose concentration in healthy dogs and in dogs with insulinomas. In the latter, blood glucose increased by 20 mg dL⁻¹ after medetomidine administration (Guedes and Rude 2013).

The mechanisms of the hyperglycaemic effects of alpha₂-agonists has not yet been clarified and the receptors involved vary according to species (Maroto et al. 1992; Ambrisko and Hikasa 2002).

In the pancreas, alpha₂-agonists decrease the insulin secretion by their action on alpha₂-adrenoceptors in the beta pancreatic cells. A study on the isolated rat pancreas has shown that alpha₂-agonists decrease insulin release by 80% while alpha₁-agonists induce only a slight decrease in insulin secretion, up to 25% (Hillaire-Buys et al. 1985; Ambrisko and Hikasa 2002). In healthy dogs, medetomidine (0.01-0.08 mg kg⁻¹) and xylazine (1-8 mg kg⁻¹) reduced insulin plasma levels dose

independently without significantly altering glucagone levels (Burton et al. 1997; Ambrisko and Hikasa 2002). In dogs with insulinomas, medetomidine ($0,005 \text{ mg kg}^{-1}$) also decreased plasmatic insulin (by 78%) (Guedes and Rude 2013).

However, the glycemic effect of α_2 -agonists may depend on different mechanisms other than the inhibition of insulin secretion and may also involve other receptors. In dogs, the α_1 -antagonist prazosin was more effective than yohimbine in antagonizing the glycemic effect of clonidine (Maroto et al. 1992). In cattle, clonidine has been reported to increase glucose release from the liver in vitro (Gorewit 1980). These studies support the glycogenolytic effect on the liver induced by α_2 -agonists by means of their affinity for α_1 -adrenoceptors. This explains the higher increase in blood glucose observed after xylazine administration as compared to medetomidine (Ambrisko and Hikasa 2002). Dexmedetomidine in dogs (0.001 mg kg^{-1}) significantly increased glucose levels only 120 minutes after IM administration. This delay of the hyperglycemic effect can be explained by the lower affinity of dexmedetomidine for the α_1 -adrenoceptors which are involved in the stimulation of glycogenesis in the liver (Restitutti et al. 2012).

Imidazoline receptors may also be involved in the regulation of the glycemic effects of this class of drugs. Imidazoline I1 agonists have been reported to regulate the human glucose metabolism (Farsang and Kapocsi 1999; Ambrisko and Hikasa 2002).

Diuretic effect

In several species, α_2 -agonist administration is associated with an increase in diuresis, and changes in urine specific gravity, pH, osmolality, creatinine concentration and electrolytic concentrations (Thurmon et al. 1978; Trim and Hanson 1986; Burton et al. 1998; Villela et al. 2005). The mechanisms of action primarily include a centrally mediated decreased secretion of the antidiuretic hormone (ADH) (Reid et al. 1979; Talukder and Hikasa 2009). In addition, the antagonism of the renal tubular effect of ADH and the α_2 -agonist mediated cardiocirculatory

alterations may contribute to their diuretic effect (Sinclair 2003). The magnitude of the diuretic effect is drug dependent and dose dependent.

In equine species and in cattle, xylazine induced a dose-dependent increase in urinary output (Thurmon et al. 1978; Thurmon et al. 1984; Trim and Hanson 1986). In ponies, xylazine 1.1 mg kg^{-1} induced a significant increase in urinary output for up to 2 hours with a peak effect between 30 and 60 minutes after administration. The treatment also induced a significant increase in potassium and sodium excretion without affecting their plasma concentrations (Trim and Hanson 1986). In mares, xylazine 0.5, 1.0, and 1.5 mg kg^{-1} induced an increase in urinary output up to 1.82, 3.93, and $5.68 \text{ ml kg}^{-1} \text{ h}^{-1}$, respectively (Thurmon et al. 1984). In cattle, the diuretic effect of xylazine 0.22 mg kg^{-1} or 0.44 mg kg^{-1} lasted up to 5 hours (Thurmon et al. 1978).

In dogs, both xylazine (0.24; 0.5; 1; 2; 4 mg kg^{-1}) and medetomidine (0.005; 0.01; 0.02; 0.04; 0.08 mg kg^{-1}) decreased ADH levels and increased urinary production dose dependently, with xylazine inducing a greater increase as compared to medetomidine (Talukder and Hikasa 2009). In this study, the diuretic effect lasted up to 4 hours after administration. Both drugs also induced a significant decrease in creatinine concentration, in osmolality and in the pH of the urine samples. The urinary specific gravity decreased in a dose dependent manner with the lowest value recorded one to three hours after administration (Talukder and Hikasa 2009).

The hypertensive response observed after the administration of α_2 -agonists may be involved in the diuretic effects; however, studies have highlighted that increased blood pressure alone cannot explain the diuretic effects. In dogs, clonidine (0.03 mg kg^{-1}) induced an increase in blood pressure and a significant decrease in plasma ADH from 10.9 ± 1.5 to $5.0 \pm 1.1 \text{ ng ml}^{-1}$. However, the administration of two α_2 -antagonists (piperoxane and phentolamine) resulted in a reversal of the pressure response without significant alteration in ADH concentration (Reid et al. 1979). On the contrary, atipamezole and yohimbine were effective in reversing the medetomidine-induced inhibition of the ADH release and the diuretic effect of medetomidine in dogs (Talukder et al. 2009). The effect of atipamezole in reversing the diuretic effect has been shown to be dose dependent and

greater as compared to that of yohimbine, probably because of its affinity for imidazole receptors (Talukder et al. 2009).

In dogs, xylazine and medetomidine administration also induced a significant increase in the atrial natriuretic peptide (ANP) (Talukder and Hikasa 2009; Talukder et al. 2009). However, in their study, Talukder and colleagues (2009) found exceptionally that atipamezole administration resulted in an additional increase in the ANP in a dose-dependent manner as compared to a reversal of the diuretic effect induced by medetomidine. Therefore, the atrial natriuretic peptide, released in response to atrial distension which, in this study, may have been associated with increased blood pressure, seems to be minimally involved in the diuretic effect induced by α_2 -agonists (Talukder et al. 2009).

Some authors have suggested not administering α_2 -agonists to patients with urinary obstructions and to take into consideration fluid loss when managing critical patients (Daunt and Steffey 2002; Sinclair 2003).

Stress response

α_2 -agonist administration has been described to reduce the perioperative release of catecholamine. In dogs, medetomidine (0.01, 0.02, 0.04, 0.08 mg kg⁻¹) and xylazine (1, 2, 4, 8 mg kg⁻¹) suppressed NA release in a dose-dependent manner. Medetomidine also reduced epinephrine release dose dependently and with a greater potency than xylazine (Ambrisko and Hikasa 2002). In bitches undergoing ovariohysterectomy who were premedicated with medetomidine, the cortisol concentration during the surgery was significantly lower than that of bitches which did not receive any premedicant drug (Benson et al. 2000). Moreover, the perioperative α_2 -agonist administration inhibited the release of cortisol through their imidazoline activity (Ambrisko and Hikasa 2002).

In another study, comparing the endocrine effects of medetomidine (0.02 mg kg⁻¹) and acepromazine (0.05 mg kg⁻¹) in bitches undergoing ovariohysterectomy, medetomidine has been

proven to be more effective in decreasing perioperative plasma catecholamine and cortisol concentrations (Väisänen et al. 2002)

Alpha₂-antagonists

Alpha₂-agonists have the advantage over other anesthetic drugs in that their effects can be reversed by the use of specific antagonists (Rankin 2015). The most commonly used antagonists in clinical practice are atipamezole, idazoxan, yohimbine and tolazoline, with atipamezole being the most selective for alpha₂-adrenoceptors and tolazoline the least selective. Atipamezole, idazoxan and yohimbine have an alpha₂:alpha₁ affinity ratio of 8526:1, 27:1 and 40:1 respectively (Virtanen et al. 1989). In an in-vitro study, Schwartz and Clark (1998) have reported that tolazoline had the lowest affinity at alpha_{2A}-, alpha_{2B}- and alpha_{2C}-, and alpha_{2D}- adrenoceptor subtypes as compared to atipamezole and yohimbine. As compared to yohimbine, atipamezole had a 100-fold affinity in alpha_{2D}-adrenoceptor subtypes, with tolazoline having a similar affinity to yohimbine (Schwartz and Clark 1998). Due to its high affinity for alpha₂-adrenoceptors and imidazoline receptors, atipamezole is the drug of choice for the reversal of dexmedetomidine and medetomidine (Virtanen et al. 1989). In addition, atipamezole has been used to reverse detomidine in horses (Skarda and Muir 1998; Hubbell and Muir 2006; Di Concetto et al. 2007). There is discrepancy among authors concerning the dosage of atipamezole required to reverse detomidine in horses, and they have reported that re-sedation after administration may occur (Di Concetto et al. 2007).

In in-vitro studies, atipamezole has been demonstrated to lack affinity for beta- adrenoceptors, for histamine receptors, muscarinic receptors, gamma-aminobutyric acid (GABA) receptors, opioid receptors and benzodiazepine receptors (Virtanen et al. 1989).

Side effects, such as hypotension, tachycardia and excitation after antagonist administration, have been reported, especially after IV administration (Sinclair 2003). Cardiovascular alterations induced by the IM administration of atipamezole in dogs is not clinically significant (Sinclair 2003). Atipamezole (0.015 and 0.03 mg kg⁻¹) IV administration in cats receiving dexmedetomidine CRI resulted in an 8% and 4% increase in the HR, respectively and in a 39% and 46% decrease in MAP,

respectively. In addition to the clinically apparent cardiovascular changes occurring, atipamezole was effective in increasing the CO and decreasing the SVR (Martin Flores et al. 2017).

*New applications of α_2 -agonists
in veterinary medicine*

Alpha₂-agonists for semen collection

Alpha₂-agonists has been used for pharmacologically induced ejaculation in-copula and ex-copula in several species (McDonnell and Love 1991; Silinski et al. 2002). Xylazine, detomidine, medetomidine and dexmedetomidine are the compounds described to date to be used for this application.

In horses, xylazine produced ejaculation within few minutes after administration without erection. McDonnell and Love (1991) have successfully described the use of xylazine (0.7 mg kg⁻¹) for semen collection in horses with ejaculatory dysfunction: in a horse with low libido and in a stallion with reduced hind limb strength (McDonnell and Love 1991). When xylazine was administered to normal stallions, ejaculation was obtained in 27% of them. Among these cases, ejaculation frequency was significantly higher when horses were left together with mares compared with trials in which horses did not undergo any pre-treatment stimulation (14%) (McDonnell and Love 1991). The adjunct of imipramine, a tricyclic antidepressant, to xylazine, induced ejaculation in the 33% of treated horses with ejaculates characterized by a higher spermatozoa concentration, higher total number of spermatozoa but a lower total volume (McDonnell and Odian 1994).

Detomidine in combination with butorphanol has been used in a white rhinoceros, to obtain ex-copula semen collection with poor results (Silinski et al. 2002). In that study various dosages of detomidine and butorphanol (total mg 8/4, 8/8, 10/4, 10/8, 14/4 respectively) or saline solution were administered randomly up to 24 trials in a single animal. The medications induced a superior and longer lasting erection and a higher number of penile contractions compared to saline solution. However, seminal fluid was collected only in 25% of medicated trials and in 21% of the control trials. In addition, only small amounts of fluids were collected. The overall motility and vitality of the spermatozoa in the samples were 60-85% (Silinski et al. 2002).

In felids, alpha₂-agonists have been included in the protocol for semen collection through urethral catheterization after pharmacological induction (Ur. Ca.P.I.). The technique was first described by

Zambelli and colleagues in tomcats and consisted in the semen collection through urethral catheterization after the administration of high dose medetomidine (0,13-0,14 mg kg⁻¹) as an alternative to electroejaculation (Zambelli et al. 2007, 2010). In felids, unlike horses, alpha₂-agonists did not induce erection and ejaculation but enhanced the sperm release into the urethra. The ultrasound examination highlighted a dilation in the prostatic urethra after alpha₂-agonists administration, corresponding to the sperm release into the urethra (Lueders et al. 2012; Kheirkhah et al. 2017). As soon as the sperm is released, the urethral catheterization allows the semen collection by means of capillarity. The technique allows the collection of good quality semen; this can be preserved by means of cryopreservation or it can be used for in vitro fertilization (Zambelli et al. 2008).

More recently the Ur.Ca.P.I. technique has been described in several wild species using different sedative drugs in combination with alpha₂-agonists (Lueders et al. 2012; Kheirkhah et al. 2017).

Lueders has described the application of the Ur.Ca.P.I. protocol in several wild species of felids including 14 animals totally: Lions -*Panthera leo*- (medetomidine 12 mg and ketamine 150 mg), Tiger- *Panthera tigris*- (medetomidine 4 mg and tiletamine-zolazepam 120 mg), African leopard- *Panthera pardus*- (medetomidine 2 mg, midazolam 10 mg and butorphanol 15 mg), Snow leopard - *Panthera uncia*- (medetomidine 1.5 mg and ketamine 100 mg), Cheetah -*Acinonyx jubatus*- (Medetomidine 2 mg and Ketamine 80 mg) and Golden cat -*Catopuma temmincki*- (Medetomidine 0.8 mg and ketamine 75 mg). In his study, the Ur.Ca.P.I. technique allowed the collection of small ejaculate volume free of urine contamination and characterized by high sperm concentrations. Only in two of the fourteen animals included the semen collection was not obtained. In addition, the authors used the sperm collected from the Asiatic golden cat to successfully perform an intrauterine artificial insemination. In African lions the Ur.Ca.P.I. technique has been applied administering medetomidine (12 mg) combined with ketamine (150 mg) (Lueders et al. 2012). In this species the urethral catheterization allowed the collection of semen samples characterized by a lower total volume and a higher sperm concentration if compared to ejaculates obtained in the same species with elettroejaculation, and with a sperm motility higher than 79.5% (Lueders et al. 2012).

In some studies, the administration of α_2 -agonists has been reported to cause urine contamination. The mechanism of action inducing this effect has not been determined yet but depends on the affinity of these drugs for α_1 -adrenoceptors. In cheetahs (*Acinonyx jubatus*) the administration of tiletamine-zolazepam ($1.17 \pm 0.14 \text{ mg kg}^{-1}$), ketamine ($1.17 \pm 0.14 \text{ mg kg}^{-1}$) and medetomidine ($0.012 \pm 0.0017 \text{ mg kg}^{-1}$) induced urine contamination when administered to provide sedation to perform electroejaculation. When dexmedetomidine ($0.01 \pm 0.0017 \text{ mg kg}^{-1}$) was used in place of medetomidine with similar doses of tiletamine-zolazepam and ketamine ($1.59 \pm 0.1 \text{ mg kg}^{-1}$ for both of them) urine contamination in semen samples was not observed (Marrow et al. 2015). In dogs, yohimbine (0.2 mg kg^{-1}) reduced significantly the spermatozoa displacement in the urinary bladder if compared to xylazine (Pineda and Dooley 1994).

α_1 and α_2 adrenoceptors have been identified in the urethra in humans and in laboratory animals (Michel and Vrydag 2006). Studies in rabbits have demonstrated that these receptors are involved in the regulation of the urethral contraction (Ueda et al. 1984). Studies on rat's *vas deferens* have demonstrated that α_1 -adrenoceptor agonists induce the contractions of the *vas deferens* through stimulation on α_{1A} -adrenoceptors subtype (Aboud et al. 1993). α_1 -adrenoceptors seems to be involved mainly in the regulation of erection and ejaculation, while α_2 seems to be involved mainly in the regulation of sexual behaviors (McDonnell and Love 1991).

The mechanism of action of medetomidine that allows the sperm release into the urethra, as described by the Ur.Ca.P.I. technique has not been clarified yet. Authors hypothesized that this effect is due to the action of α_2 -agonists on the adrenoceptors in the smooth muscles of *vas deferens*. However, the distribution of α adrenoceptor in urethra and *vas deferens* has not been described yet in these species and is the topic of ongoing researches (personal unpublished results).

High doses of α_2 -agonists increase the duration of the sedative effects but also the incidence of cardiovascular side effects. However, in more recent studies, authors have observed that lower doses of medetomidine (0.05 mg kg^{-1}) administered to healthy cats for the Ur.Ca.P.I technique

allowed the collection of semen characterized by lower quality in terms of volume, sperm concentration, percentage of motility and progressive motility (Zambelli et al. 2011; Cunto et al. 2015).

Doses of medetomidine used to perform the Ur.Ca.P.I. are higher than those commonly used in the clinical practice and significant cardiovascular alterations have already been described after administration of lower doses (Sinclair 2003). To the author knowledge there are no studies evaluating the hemodynamic alterations induced by high dose medetomidine in cats.

In the study *Non-invasive evaluation of the haemodynamic effects of high-dose medetomidine in healthy cats for semen collection* the hemodynamic alterations induced by medetomidine 0,013 mg kg⁻¹ administered to healthy male cats undergoing semen collection using the Ur.Ca.P.I. protocol as described by Zambelli and colleagues (2007, 2010) have been investigated. The hemodynamic alterations were evaluated based on the clinical examination, on the blood pressure measurements using a Doppler device and on the echocardiography.

The study has been published in the Journal of Feline Medicine and Surgery (No 18, 2016; pages: 337-343). The final draft post refereeing is reported. Changes may have been made on this work since it was submitted for publication.

**NON-INVASIVE EVALUATION OF THE HAEMODYNAMIC EFFECTS OF HIGH DOSE
MEDETOMIDINE IN HEALTHY CATS FOR SEMEN COLLECTION**

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Abstract

Objectives – To non-invasively assess the cardiovascular effects of high dose medetomidine on healthy male cats undergoing semen collection.

Methods – Haemodynamic evaluations were assessed on the basis of clinical examination, systemic arterial blood pressure and transthoracic echocardiography. Eight male domestic short hair client-owned cats were sedated with a bolus of medetomidine intramuscularly (0.13 mg/kg), and semen collection was performed. A second transthoracic echocardiography and systemic arterial blood pressure measurement were carried out 15 minutes after sedation. At the end of the examination, the patients received an atipamezole bolus (0.3 mg/kg) intramuscularly.

Results–The cats were deeply sedated, relaxed and laterally recumbent during the entire procedure. No rhythm abnormalities were observed during the exams and no significant increase in systemic arterial blood pressure was recorded. The heart rate dropped from 200 ± 33 to 92 ± 13.1 beats per min after sedation. There was a significant increase in left ventricular dimensions and the left atrial area. The parameters of left ventricular systolic function were reduced, as were systemic and pulmonary cardiac outputs. Peak diastolic wave velocities were significantly reduced while isovolumic contraction and relaxation of the left ventricle were prolonged. Aortic valve insufficiency was recorded for all cats while mitral valve insufficiency was noticed in five cats. None of the subjects developed systolic anterior motion.

Conclusion and relevance – The protocol allowed to collect good semen samples in healthy cats. However, high dose medetomidine induces significant haemodynamic effects on the feline heart, mainly due to a reduced heart rate, an increased cardiac preload and impaired systolic function. The animals recovered from the anaesthesia after antagonist administration, without showing any clinically relevant consequences.

Introduction

Medetomidine is a highly selective α_2 -adrenoreceptive agonist; it is widely used in veterinary medicine to induce sedation, analgesia and muscle relaxation.¹⁻³ Medetomidine is used alone or in combination with other drugs for minor surgical and interventional procedures and, if used for premedication, reduces the amount of drugs necessary to induce and maintain general anaesthesia.^{1,3} In clinical practice, the dose used in cats varies from 0.02 to 0.08 mg/kg.^{1,3} The sedative and analgesic effect, their depth and duration are dose dependent and can be completely reversed by using the antagonist atipamezole.²⁻⁴

Alpha-2-adrenoreceptor activation produces effects on the central nervous system, desirable for patient anaesthetic control and, at the same time, it also has several cardiovascular effects. Due to peripheral arterial vasoconstriction, medetomidine induces responsive bradycardia mediated by ventricular baroreceptors. This effect is potentiated by the action of the drug on the central nervous system with subsequent reduced sympathetic tone.¹⁻⁷ Cardiac output (CO) is typically reduced when medetomidine is administered in a healthy animal as a consequence of increased systemic vascular resistance (SVR) and reflex bradycardia.^{1,3,4,8}

In dogs, medetomidine is reported to induce some electrocardiographic alterations, such as enhanced sinus arrhythmia, sinus bigeminy and atrioventricular blocks.^{1,3,9} In cats with hypertrophic obstructive cardiomyopathy due to the systolic anterior motion (SAM) of the mitral valve, medetomidine is reported to reduce the left ventricular outflow tract gradient and to resolve the dynamic stenosis.⁸

Alpha-2-adrenoreceptive agonists and medetomidine can also be used for semen collection. In horses, erection and ejaculation can be artificially produced by administering xylazine or imipramine.¹⁰ In cats, medetomidine induces a myorelaxant effect on the deferent duct, leading to sperm ejection and the release of spermatozoa within the urethra.^{11,12} This method of semen collection in the feline is feasible and apparently safe; however, the dose of medetomidine needed to produce a good semen sample is much higher than the dose reported for anaesthetic and pain management procedures, and varies in cats between 0.13 and 0.14 mg/kg.^{12,13} The cardiovascular effect of such a dose of medetomidine in a population of feline patients has never been evaluated.

The aim of the present study was to non-invasively assess the cardiac and vascular sequelae of high dose medetomidine in a group of healthy male cats undergoing semen collection.

Materials and methods

Animals

Privately owned intact male cats undergoing semen collection at the small animal reproduction service of the Veterinary Teaching Hospital were used for the study. All the owners were informed about the study and were asked to sign a written informed consent form. The experiment was conducted in accordance with the provisions of European Economic Community Council Directive 86/609, adopted by the Italian Government (D.L. 27/01/1992 n° 116). The study was approved by the local and national ethical committees (SVC 43/2013).

All the animals underwent a complete clinical examination by an anesthetist and a cardiologist in order to exclude cats having major systemic abnormalities or cardiac murmurs. The patients were included if no clinical signs (gastrointestinal, neurological, respiratory or cardiovascular disorders) were detected. Thus, all the animals needed to be classified as I according to the American Society of Anesthesiologists' (ASA) classification of physical status. The patients were hospitalized for the time required by the procedure and until complete recovery from sedation. At the end of the protocol, all the animals underwent a second clinical examination, and were dismissed in accordance with the judgment of the anesthetist.

Sedation protocol and semen collection

Each patient received a single bolus of medetomidine (0.13 mg/kg; Sedastart, ESTEVE S.p.A) intramuscularly (IM) and was placed into a cat carrier sheltered from light and noise but strictly monitored in order to evaluate sedation status and possible side effects. After 15 mins, the patients were taken out of the carriers and cardiovascular examination was performed as described below.

When all the procedures were completed, semen collection was done by Urethral Catheterization After Pharmacological Induction (UR.C.A.P.I.), as previously described;^{12,13} the semen collection required approximately 5 minutes. During the entire procedure, the cats received oxygen supplementation through an anaesthesia mask. At the end of the examination, the patients received an atipamezole bolus (0.3 mg/kg IM, Sedastop, ESTEVE S.p.A.). After 10 mins from the injection of the antagonist the grade of sedation and the heart rate were evaluated.

After animal sedation, the entire procedure took approximately 30 minutes.

Cardiovascular examination

Haemodynamic evaluations, assessed on the basis of clinical examination (femoral pulse evaluation, mucous membranes and capillary refill time), systolic arterial pressure (SAP) and transthoracic echocardiography were carried out before sedation with awake, manually restrained cats, and then repeated 15 mins after medetomidine administration.

Systolic arterial blood pressure was measured using a Doppler device (Minidrop ES-100 VX, Hadecco) as reported by Binns et al.¹⁴ In detail, the Doppler probe was placed over the common digital branch of the radial artery on the palmar aspect of the foot. The hair was clipped before placing the probe. Ultrasound transmission gel (Aquasonic 100, Parker Laboratories, INC.) was placed between the probe and the skin to improve ultrasonic contact, and the volume of the Doppler machine was adjusted to obtain a clearly audible signal. The cuff was inflated until flow sounds were no longer audible, and then gradually deflated until clear flow sounds became audible. The manometer reading at the reappearance of flow sounds was recorded as the SAP as described by Jepson et al.¹⁵ Five repeated measurements were carried out; the highest and the lowest values were excluded, and the mean value was calculated.

Transthoracic echocardiography was performed by the same experienced operator (MBT) using an ultrasound unit (M7Vet ultrasound unit, Shenzhen Mindray Bio-Medical electronics Co.) equipped with a multifrequency phased array probe using second harmonic settings (7-3 MHz) and continuous ECG tracing. All echocardiographic examinations were carried out with the cats in both right and left

lateral recumbency, using a dedicated table, and following a standard approach as described elsewhere.¹⁶ Complete M-Mode, 2-dimensional, color, pulsed wave and continuous wave Doppler analysis were carried out. The images and videoclips were stored in the internal hard disk memory of the ultrasound unit and used subsequently for off-line analysis. The echocardiographic variables measured and calculated for this study are listed in Table 1. All the measurement criteria and the formulae used to calculate some variables were obtained from the veterinary literature and are detailed elsewhere.¹⁷

Statistical analysis

Data are reported as means and SD. Descriptive statistical analyses were carried out using a commercial software program (Microsoft Excel, Microsoft Corporation). Continuous data were tested for normality using a D'Agostino-Pearson test. Haemodynamic variables before and after medetomidine administration were compared using a paired t test for repeated measurements. $P < 0.05$ was considered significant. This part of the analysis was run using dedicated software (Prism 5, GraphPad Software Inc.).

Results

Animals and sedation

Eight cats were enrolled in the study on the basis of the above-mentioned criteria. All animals were domestic short hair cats, with an age ranging from 6 to 96 months and having a mean body weight of 3.6 ± 0.9 kg.

All patients vomited gastric secretions once within 15 mins after medetomidine injection. All the cats were deeply sedated, relaxed and laterally recumbent during the entire procedure. Semen collection was feasible in all animals. Atipamezole administration produced a visible decrease in the quality of sedation within 10 mins after injection; sternal recumbency was achieved within 20 mins in all cats, and complete recovery without any grade of sedation was recorded; the discharge was possible not more than one hour after the end of the procedure. The owners did not notice any abnormalities at home.

Cardiovascular examination

Arterial blood pressure and echocardiographic examination were performed before and after sedation in all cats. No rhythm abnormalities were observed during the exams. All the recorded haemodynamic variables are listed in Table 1. Variables with a statistical difference before and after sedation are indicated. Heart rates dropped significantly from 200 to 92 bpm (as a mean value) ($P<0.01$) after medetomidine administration. Systolic arterial pressure increased after medetomidine administration, but the difference was not statistically significant. Preload increased, as expressed by increased left ventricular diastolic dimensions and atrial area, while left ventricular systolic function, and its related variables, including CO, appeared reduced due to the medetomidine effect (Fig 1). Some diastolic variables, such as E wave, representing the peak velocity of the early left ventricular diastolic refill wave, and A wave, the peak velocity of the late left ventricular refill wave due to active atrial contraction, and isovolumic relaxation time (IVRT) were also affected in terms of reduction of the first two and an increase in the last one. Left ventricular wall thickness and great vessel diameter did not change before and after sedation. Only left ventricular posterior wall (LVPWs) thickness in systole decreased after sedation. Interestingly, aortic valve insufficiency was recorded for all cats while mitral valve insufficiency was noticed in 5 out of 8 cats as an effect of the medetomidine. None of the subjects developed SAM.

Discussion

In the present study, we echocardiographically evaluated the haemodynamic effects of high dose medetomidine in healthy cats undergoing semen collection using UR.C.A.P.I.^{12,13}

The main finding observed was that high dose medetomidine induces significant haemodynamic effects on the feline heart.

The semen collection using UR.C.A.P.I did not produce any pain to cats. The introduction of a urinary 3F tomcat catheter (Portex Jackson Cat Catheter, Jorgensen Laboratories) in the urethra for semen

collection did not evoke any response in cats and changes in cardiac or respiratory frequency were not recorded.¹²

The procedure was feasible in all animals, and the patients recovered within 30 minutes after the end of the protocol, and only vomiting was observed as adverse effect. However, several haemodynamic parameters, as assessed by transthoracic echocardiography, changed significantly after sedation, leading to a reduced heart rate (HR), increased preload and reduced systolic function of the left ventricle (LV). As an expression of this, CO also decreased significantly after sedation, mainly due to the reduction in HR.

Some of these findings have previously been described in cats receiving low doses (0.02 mg/kg) of medetomidine.⁴ Cardiac output tends to be reduced by 50 to 70% from the starting values, both due to the bradycardia effect and suppressed myocardial contractility. This degree of reduction is similar between our study and previous studies where lower doses of medetomidine were tested.^{2,4} Systemic blood pressure tended to increase in our study, but no statistical difference was observed. In previous studies, the SAP did not show any relevant variation during the first 15 mins after administering the medetomidine, but there was a progressive decrease later on.^{4,18} In the study of Lamont et al.,⁴ only mean and diastolic systemic pressure increased 15 mins after drug administration. In our study, we were only able to measure the systolic values using a Doppler device; therefore, our accuracy in detecting pressure variations during monitoring could have been underpowered. In fact Jepson et al¹⁵ found that the Doppler device in cats yields superior results than an oscillometric machine for determination of systolic blood pressure only, while the determination of the diastolic blood pressure is not reliable and more difficult to obtain.

Systolic function of the LV as assessed by fractional shortening (FS), ejection fraction (EF), end systolic volume (ESV), left ventricular internal diameter in systole (LVIDs), and forward aortic and pulmonary flows (VTIs) were significantly reduced in the cats in the present study after medetomidine was administered. These parameters are not HR dependent as is CO, and their reduction represents clear evidence of suppressed systolic function and therefore likely reduced

contractility of the ventricle. The clearest explanation for this is the abrupt increase in systemic vascular resistance mediated by the medication, which induced an increase in afterload and subsequent depressed contractility due to a baroreceptor reflex.⁸

On the other hand, in our cats, a significantly increased pre-load was found in response to the medetomidine administration. Both left ventricular and atrial dimensions increased, as seen by increased LV internal diameter and volume in diastole (LVIDd and EDV) and increased left atrium (LA) area. Lamont et al ⁴ recorded an increase in pulmonary capillary wedge pressure (PCWP) in cats sedated with medetomidine. Similarly, Pypendop and Verstegen reported a transient increase in PCPW in dogs after intravenous (IV) administration of medetomidine, and this effect appeared to be dose dependent.⁵ Since PCPW approximates LV preload, a possible explanation for this finding could be a transient response to an acute circulatory stasis related to bradycardia and to an increased LV afterload, resulting in blood stasis in the pulmonary capillaries. Another explanation could be the reduction in myocardial compliance secondary to drug-related decreased myocardial perfusion.^{4,5} Similarly, an increase in the SAP expressed by an increase in central venous pressure has been described in dogs receiving medetomidine as an expression of reduced venous capacitance and CO, and subsequent blood stasis on the venous side.⁵

Some LV diastolic variables are known to be affected by HR, such as left ventricular ejection time (LVET), IVRT, isovolumic contraction time (IVCT), and LV inflow waves. This could have affected the significance of our results since we found a statistical difference for some of these variables in our cohort of cats before and after medetomidine administration. However, in a study conducted on healthy cats, the authors concluded that, even if a weak correlation is present between HR and mitral valve (MV) E and A wave peak velocity, and IVRT, this effect has only minimal influence on the haemodynamic variables, and it is unlikely to produce clinical relevance.¹⁹

Aortic and MV insufficiency were highly prevalent in the cats in our study after sedation. This might partially be explained by the increased blood pressure which led to stress on the aortic valve with subsequent mild leakage during diastole. On the other hand, the LV dilation could have produced a

mitral annulus stretch with the subsequent loss of complete closure of the mitral leaflets during LV contraction.

In our study, the cats were judged to be healthy, and recovered completely after sedation. At the end of the study vomiting was the only adverse clinical effect seen after medetomidine administration. Vomiting could be a cause of mortality in sedated animals because of aspiration.

However, in our cohort, it consisted only of small quantity of gastric secretions and the mouth was inspected to exclude the presence of foreign material. Other relevant clinical disturbances were not reported by the anaesthetist and the owners. This suggests that, despite the considerable effect on the cardiovascular function we observed, these changes are not clinically relevant and dangerous in healthy cats. Moreover similar negative effects on the cardiovascular system have already been observed in cats even with conventional doses of medetomidine, meaning that some cardiovascular sequelae are intrinsic in the mechanism of action of the molecule and are not necessarily dose related. It should however be noted that, in another study conducted on cats, such significant echocardiographic changes in heart function were not detected when using a standard dose of medetomidine.⁸ Whether high dose medetomidine might significantly impair the cardiovascular system, leading to subtle consequences for the animal, cannot be assessed on the basis of our study. Some limitations must be highlighted in the present study. First cats were not re-examined after administration of atipamezole to evaluate the presence of residual myocardial damage or cardiovascular alterations secondary to medetomidine injection. However cats were judged to be clinically normal by the anaesthetist at the time of discharge from the hospital, and owners didn't recognize any abnormality at home. Second, even if cats were considered healthy on the basis of baseline cardiovascular examination, a subtle myocardial alteration could not be excluded for sure, since some cardiomyopathies in cats might be missed until they produce hemodynamic sequelae. Third, LV volumes were calculated using also the Teicholz formula, that has not been validated in cats, while other 2-dimensional values might be more appropriate in this specie.²⁰ However, Teicholz method was just used as an arbitrary way to evaluate the drug induced cardiovascular alterations in

the cats of the present study; and these alterations remained statistically relevant even when using different techniques to measure CO (i.e. Doppler derived SV) or just measuring the LV diameters during systole and diastole. Finally, one single dose of medetomidine was used. This was done since the purpose of our study was to evaluate this specific sedative protocol that has been standardized in previous studies as a method for semen collection in cats.^{12,13}

The cats used in the present study were judged to be healthy on the basis of a clinical examination and baseline echocardiography. The medetomidine dose used seemed to be well tolerated. However, it cannot be ruled out that a significant negative effect on cardiac function might be produced in cats with heart disease. A careful cardiovascular examination and echocardiography should be carried out before sedation with high dose of medetomidine.

Conclusion

In the present study, the cardiovascular effects of high dose medetomidine (0.13 mg/kg) in intact male cats undergoing semen collection were evaluated non-invasively. Significant modification in both systolic and diastolic cardiac function and in the SAP were observed. The animals were stable during the procedure and completely recovered from the anaesthesia after antagonist administration. Whether this sedative protocol might be harmful in cats affected by heart disease remains to be clarified. However, since medetomidine can significantly impair cardiac function, a cardiovascular examination and echocardiography should be performed before the procedure and the protocol should be reserved to cats assessed as ASA I only.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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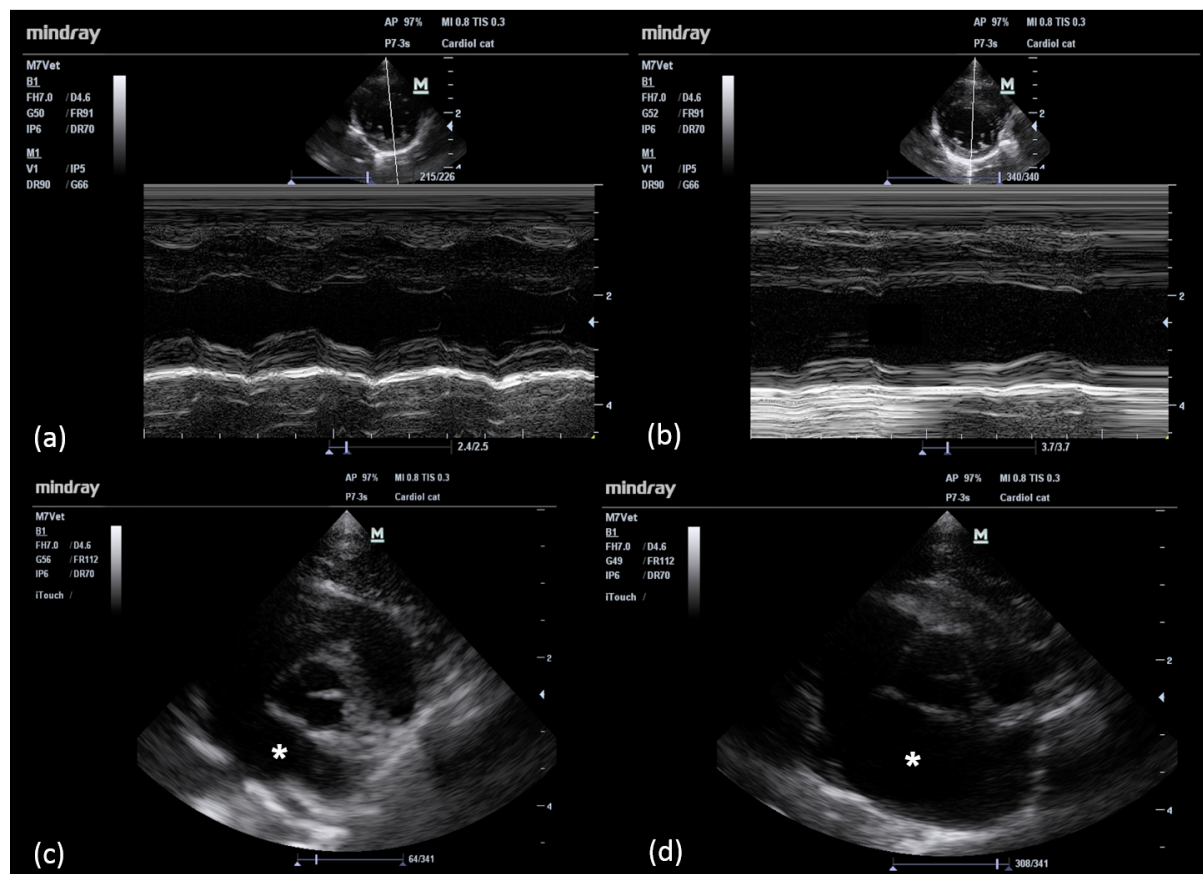


Figure 1. Echocardiographic images of two cats enrolled in the study before (left panels) and after (right panels) the intramuscular administration of high dose medetomidine (0.13 mg kg^{-1}) for sperm collection. In the upper panels, a 2-dimensional guided M-mode analysis of the left ventricular function over time obtained from one cat enrolled in the study is displayed. Note the ventricular diameter before (a) and after (b) medetomidine administration. There is a clear decrease in heart rate and an increase in both diastolic and systolic dimensions with a subsequent reduction in systolic function. In the bottom panels 2-dimensional images of the heart base and left atrium (asterisk) of another cat enrolled in the study group are shown. An obvious difference in the left atrial area is visible between the baseline state (c) and after sedation (d).

	Baseline	Under sedation	P value
Heart rate (bpm)	200±33	91.6±13.1	p<0.001
Systolic blood pressure (mmHg)	136±12	150±25	p=0.216
M-mode derived variables			
IVSd (mm)	3.9±0.3	4.1±0.7	p=0.562
LVIDd (mm)	14.1±1.2	15.3±1.9	p=0.024
LVPWd (mm)	3.9±0.5	3.8±0.7	p=0.695
IVSs (mm)	6.2±0.9	5.2±1.0	p=0.106
LVIDs (mm)	7.3±0.6	11.1±1.9	p<0.001
LVPWs (mm)	6.4±0.8	5.2±0.8	p=0.001
FS (%)	47.9±3.7	27.6±7.1	p<0.001
EF (%)	82.3±2.9	56.8±10.8	p<0.001
EDV (ml)	5.2±1.2	6.6±2.1	p=0.022
ESV (ml)	0.9±0.2	2.9±1.2	p<0.001
SV (ml)	4.3±1.0	3.7±1.2	p=0.055
CO LV (L/min)	0.9±0.3	0.3±0.1	p<0.001
Doppler derived variables			
Aortic velocity (cm/sec)	102.9±24.8	65.2±12.6	p=0.001
Aortic VTI (cm)	8.7±1.6	7.8±1.2	p=0.073
Aortic diameter (mm)	8.2±1,3	8.2±0.8	p=0.896
Aortic CSA (cm2)	2.1±0.7	2.1±0.4	p=1.000
SV LV (ml)	1.9±0.7	1.7±0.5	p=0.270
CO LV (L/min)	0.4±0.1	0.2±0.04	p<0.001

LVET (msec)	126.5±20.8	175±11.8	p=0.002
Pulmonic valve VTI (cm)	10.0±1.4	6.5±1.4	p=0.001
Pulmonic valve diameter (mm)	7.3±0.9	7.8±1.0	p=0.055
Pulmonic valve CSA (cm ²)	1.7±0.4	2.0±0.5	p=0.081
SV RV (ml)	1.7±0.5	1.3±0.5	p=0.002
CO RV (L/min)	0.3±0.1	0.1±0.03	p<0.001
MV E wave peak velocity (cm/sec)	82.0±16.5	54.0±16.8	p=0.002
MV A wave peak velocity (cm/sec)	70.8±22.5	31.6±6.4	p=0.003
MV VTI (cm)	6.7±0.7	7.1±0.6	p=0.254
E wave DT (msec)	65.4±12.6	76.5±21.7	p=0.222
E:A	1.2±0.3	1.8±0.7	p=0.117
IVRT (msec)	34.9±7.8	70.1±7.5	p<0.001
IVCT (msec)	23.1±10.7	53.0±12.8	p<0.001
LA diameter (mm)	11.3±1.2	12.0±1.6	p=0.262
LA area (mm ²)	17.7±2.9	21.4±5.0	p=0.029
LA:Ao	1.4±0.3	1.5±0.1	p=0.620
Presence of SAM	0/8	0/8	
Presence of aortic insufficiency	0/8	8/8	
Presence of mitral valve insufficiency	0/8	5/8	

Table 1: Haemodynamic variables in 8 male cats sedated with high dose medetomidine (mean±SD). Ao: aorta; CO: cardiac output; CSA: cross sectional area; DT: deceleration time; EF: ejection fraction; EDV: end-diastolic volume; ESV: end-systolic volume; FS: fractional shortening; IVCT: isovolumic contraction time; IVRT: isovolumic relaxation time; IVSd: interventricular septal thickness in diastole; IVSs: interventricular septal thickness in systole; LA: left atrium; LV: left ventricle; LVET: left ventricular ejection time; LVIDd: left ventricular internal diameter in diastole; LVIDs: left ventricular internal diameter in systole; LVPWd: left ventricular posterior wall thickness in diastole; LVPWs: left ventricular posterior wall thickness in systole; MV: mitral valve; RV: right ventricle; SAM: systolic anterior motion of the mitral valve; SV: stroke volume; VTI: velocity time integral.

Dexmedetomidine infusion during surgery in two dogs undergoing adrenalectomy for a suspicion of a pheochromocytoma

Introduction

Pheochromocytoma (PHEO), as defined by the World Health Organization (WHO), is a rare tumor originating from the chromaffin cells of the adrenal medulla and, in dogs, it accounts for up to 0.1% of all canine tumors (Reusch 2015). Chromaffin cells are involved in the production and storage of dopamine, norepinephrine and epinephrine which are released with the concurrent activation of the sympathetic nervous system (Reusch 2015).

The embryological origin of the chromaffin cells is similar to that of the sympathetic post-ganglionic neurons: chromaffin cells are modified sympathetic neurons which synthesize, store and secrete catecholamines upon stimulation. Both adrenergic and noradrenergic chromaffin cells have been identified; these cells, on the basis of the stimulus, secrete epinephrine or norepinephrine. Authors have hypothesized the existence of a feedback mechanism regulating the catecholamine release, similar to that of sympathetic neurons. In a study using α_2 -knock-out mice, authors have found that α_{2A} -deficient mice had higher norepinephrine concentrations while α_{2C} -deficient mice had higher adrenaline concentrations (Brede et al. 2003). Therefore, the authors identified α_{2C} as the main adrenoceptor subtype involved in the regulation of epinephrine release in the adrenal medulla (Brede et al. 2003). In addition, only the mRNA of the α_{2C} -adrenoceptor subtype has been identified in isolated chromaffin cells from Wild Type mice (Brede et al. 2003). Similarly, in the adrenal glands isolated from mice, the α_{2C} - has been identified as the main adrenoceptor subtype involved in the regulation of the feedback inhibition of the epinephrine secretion by the adrenal gland (Moura et al. 2006). In the same study, the authors have demonstrated that

medetomidine reduced the outflow of epinephrine and norepinephrine in a concentration dependent manner (Moura et al. 2006).

Imidazole receptors may also be involved in the regulation of catecholamine release from the adrenal glands. In isolated bovine adrenal glands, the imidazole α_2 -agonist clonidine inhibited concentration dependently the catecholamine secretion induced by carbachol, an acetylcholine synthetic agonist; the administration of non-imidazole α_2 -antagonists did not have any effect on the secretion of catecholamine induced by the carbachol (Powis and Baker 1986). In a similar study regarding isolated bovine chromaffin cells, imidazole agonists inhibited the acetylcholine-evoked release of catecholamine from the adrenal medulla. This inhibition was antagonized by α_2 -antagonists with affinity for imidazole receptors (tolazoline and cimetidine) but not by nonimidazole α_2 -antagonists (Ohara-Imaizumi and Kumakura 1992)

In patients with PHEO, neoplastic cells secrete elevated quantities of catecholamines which influence blood pressure, heart rate (HR) and the metabolism in human and small animal patients; clinical signs are related to the catecholamine release and depend on their frequency of secretion. Moreover, the mass effect of the tumor and its invasiveness can influence the clinical presentation (Barthez et al. 1997).

Adrenalectomy is the treatment of choice for PHEO in dogs (Herrera et al. 2008). However, a report regarding this species has recognized the mortality rate to be 11.7% among dogs undergoing surgical treatment as a result of cardiovascular complications (Gilson et al. 1994; Barthez et al. 1997).

In humans, the goal of perioperative stabilization is to block the effects of catecholamine by means of the administration of phenoxibenzamine, a non-competitive α_1 -adrenoceptor blocker which acts by blocking the response to circulating catecholamines without inhibiting their synthesis (Reusch 2015). Its administration helps in reversing the vasoconstriction induced by catecholamines and prevents intraoperative pressure fluctuations.

In dogs, the administration of phenoxibenzamine for two weeks before surgery increased the survival rate with respect to untreated dogs (48% and 13%, respectively) but it was not completely effective in reducing the incidence of cardiovascular complication associated with PHEO ablation (Herrera et al. 2008). In fact, in the study of Herrera and colleagues, both treated and untreated dogs experienced hypertension, hypotension or arrhythmia. Postoperative arrhythmias, disseminated intravascular coagulation, acute respiratory distress syndrome or pancreatitis have been reported as causes of death even in dogs treated with phenoxybenzamine (Herrera et al. 2008)

In human medicine, several studies have described the perioperative administration of dexmedetomidine for the anesthetic management of patients undergoing PHEO resection, alone or in combination with remifentanyl (Wong and Cheung 2004; Schumann and Hudcova 2010; Khetarpal et al. 2014; Singh and Singh 2014). In a patient with a suspicion of PHEO, dexmedetomidine 0.001 mg kg⁻¹ infused over a minute suppressed the levels of norepinephrine by 49.42% (Singh and Singh 2014). Some preclinical studies have also suggested that dexmedetomidine infusion might contribute to the maintenance of good hemodynamic stability in these patients (Schumann and Hudcova 2010; Khetarpal et al. 2014). Schumann and Hudcova (2010) have described the anesthetic management of a patient with PHEO undergoing a surrenalectomy using a loading dose of dexmedetomidine (0.001 mg kg⁻¹) followed by an infusion at 0.0002- 0.0005 mg kg⁻¹h⁻¹. In this case the alpha₂-agonist prevented significant alteration in HR during mass manipulation as well as preventing fluctuating pressure values. In another study, similar doses of dexmedetomidine in combination with sevoflurane did not prevent significant increases in blood pressure and HR during mass manipulation but recovery from the anesthesia was uneventful (Khetarpal et al. 2014).

The aim of this study was to describe the perioperative anesthetic management with dexmedetomidine infusion in dogs undergoing celiotomy for adrenalectomy for a suspicion of pheochromocytoma.

Case 1

A 10-year-old mixed breed female dog (weight 7.2 kg) was presented to the Veterinary Teaching Hospital of the University of Bologna as it had been vomiting for 10 days.

On admission, the dog's heart rate was 160 beats min⁻¹, respiratory rate (f_R) was 28 breaths min⁻¹ and indirect arterial blood pressures measured with an oscillometric device were: 170 (systolic arterial pressure-SAP), 90 (diastolic arterial pressure-DAP) and 117 (mean arterial pressure-MAP) mmHg. The hemato-biochemical blood profile was within the normal range.

Ultrasound examination showed a heterogeneous mass (3.5 x 5 cm) which was diagnosed as a right adrenal gland increased in volume. An adrenocorticotrophic hormone (ACTH) stimulation test was negative, and catecholamine plasma concentrations were normal with the exception of metanephrine: 156 ng ml⁻¹ (range 5.4-143.1 ng ml⁻¹). Suspicion of a pheochromocytoma was hypothesized.

An exploratory laparotomy was scheduled fifteen days later; in this period, the dog was treated with phenoxybenzamine (0.6 mg kg⁻¹, twice daily). Prior to the procedure, the owner was asked to sign a written informed consent form permitting the surgical and medical treatment.

On the day of surgery, the HR was 158 beats min⁻¹, the f_R was 24 breaths min⁻¹ and blood pressure measured using an oscillometric device was 170 (SAP), 68 (DAP) and 102 (MAP) mmHg. Premedication consisted of dexmedetomidine (Dexdomitor, Elanco Animal Health; Greenfield, MA) 0.001 mg kg⁻¹ administered intramuscularly (IM). The dorsal pedal artery was cannulated immediately after premedication of the patient in order to measure invasive blood pressure. Before anesthesia induction, the HR was 108 beats min⁻¹ with rhythmic pulse, the f_R was 12 breaths min⁻¹ and the invasive blood pressures was: 140 (SAP), 78 (DAP) and 98 (MAP) mmHg. General anesthesia was induced 15 minutes after premedication with propofol (Propofol Kabi, Fresenius Kabi; Bad Homburg, Germany) administered intravenously (IV) to effect (2.5 mg kg⁻¹) and, after intubation, general anesthesia was maintained with isoflurane (Isoflo, Abbott Laboratories Ltd; Chicago, USA) vaporized in 100% oxygen (1 L min⁻¹). The dog was mechanically ventilated in order to maintain an end-tidal carbon dioxide partial pressure of 45 ± 5 mmHg. Dexmedetomidine and

remifentanyl (Ultiva, GlaxoSmithKline Manufacturing Spa, Parma, Italy) were administered IV via continuous infusion at $0.0005 \text{ mg kg}^{-1}\text{h}^{-1}$ and $0.0003 \text{ mg kg}^{-1}\text{min}^{-1}$, respectively, started at the moment of induction. Lactated Ringer's solution (Ringer lattato, ACME; Reggio Emilia, Italy) ($10 \text{ mL kg}^{-1}\text{h}^{-1}$) was administered IV throughout the anesthesia. Constant intraoperative monitoring was carried out with f_R , an electrocardiogram (lead II), pulse rate and hemoglobin saturation measured using a pulse oximeter, measurement of in- and expired gas concentrations, esophageal temperature and invasive blood pressures derived from a multiparametric monitor (Datex-Ohmeda- S3; Datex-Ohmeda Inc; Madison, USA).

Total anesthesia time was 160 minutes; the surgical manipulation of the mass lasted 72 minutes. An end-tidal isoflurane concentration of 1-1.2% was necessary to maintain anesthesia throughout the surgery.

At induction, no remarkable hemodynamic changes were recorded. The mean HR for the entire period of anesthesia was 86 (range 70-124) beats min^{-1} . The mean arterial pressure was 72 mmHg (range 65-85 mmHg) (Figure 1). An increase in HR ($124 \text{ beats min}^{-1}$) and blood pressure (170 mmHg SAP, 85 mmHg MAP and 43 mmHg DAP) related to the surgical manipulation of the mass were detected. After tumor removal, the blood pressure decreased progressively up to a SAP of 120 mmHg. The mean end-tidal carbon dioxide partial pressure was 44 ± 3 (range 41–48) mmHg during anesthesia. At the end of the surgery, the isoflurane was discontinued, and the dog regained complete consciousness in 25 minutes. After extubation, dexmedetomidine and remifentanyl infusions were stopped; postoperative analgesia was provided by methadone (Eptadone, Molteni Farmaceutici; Florence; Italy) every four hours for three days and then by buprenorphine (Temgesic, RB pharmaceuticals Limited; Slough, UK) ($0.015 \text{ mg kg}^{-1} \text{ IM}$) every 8 hours until the dog was discharged.

The dog was discharged five days later with a histological and immunohistochemical diagnosis of adenocarcinoma.

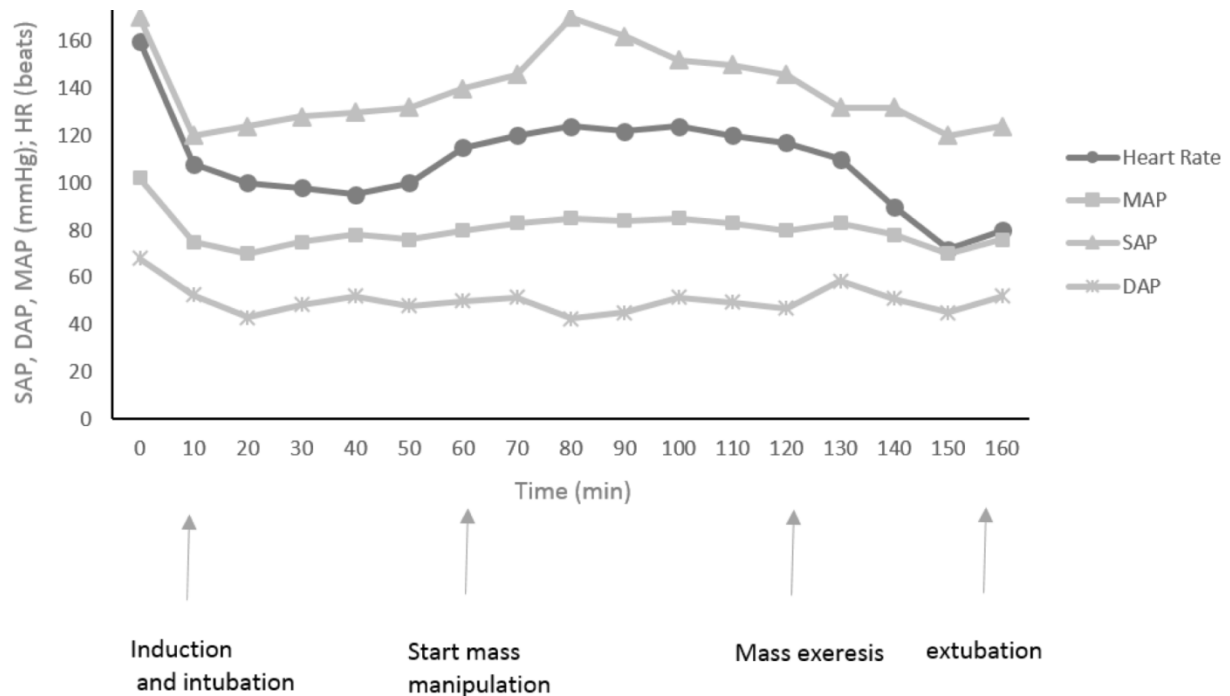


Figure 1- Cardiovascular parameters for CASE 1 recorded before sedation, at induction and intubation, during mass surgical manipulation (T60-T120) and at the time of extubation (T160): Heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP) mean arterial pressure (MAP). Dexmedetomidine ($0.0005 \text{ mg kg}^{-1}\text{h}^{-1}$) and remifentanyl ($0.0003 \text{ mg kg}^{-1}\text{min}^{-1}$) were administered by constant rate infusion throughout the anesthesia.

Case 2

A 5-years-old mixed breed male dog (weight 6.4 kg) was presented to the Veterinary Teaching Hospital of the University of Bologna with an history of vomiting and diarrhea for six months. The ultrasound and the computed tomography (CT) examinations revealed a right adrenal gland increased in volume (2.0 x 1.0 cm) and an invasion of the vena cava (1.5 x 1.0 cm). At the first clinical evaluation, the pulse rate was 68 bpm; the blood pressure was evaluated with an oscillometric device (PetMap) and the following values were recorded: 163 (SAP), 102 (DAP) and 118 MAP. The blood chemistry was in the normal range but the urinary Normetanephrine: creatinine ratio was increased (201.76; range 25.2-120.1). A suspicion of pheochromocytoma was hypothesized. The dog was treated with phenoxybenzamine for three weeks before surgery (adrenalectomy); the therapy induced a regression of the gastrointestinal symptoms and a decrease in blood pressure. Prior to the procedure, the owner was asked to sign a written informed consent form permitting the surgical and medical treatment.

On the day of surgery, the dog's HR was 100 beats min^{-1} and the blood pressure was 121 mmHg (SAP), 87 mmHg (DAP) and 96 mmHg (MAP). The patient was premedicated with dexmedetomidine 0.001 mg kg^{-1} administered IM. Propofol (4 mg kg^{-1} IV) was used for induction until endotracheal intubation was achieved, and general anesthesia was maintained with isoflurane in oxygen and air (50:50). During the procedure, dexmedetomidine (0.0005 mg $\text{kg}^{-1}\text{h}^{-1}$) and remifentanil (0.0003 mg $\text{kg}^{-1}\text{min}^{-1}$) CRIs were administered starting soon after induction. Lactated Ringer's solution (10 mL $\text{kg}^{-1}\text{h}^{-1}$) was administered IV throughout the anesthesia. The intraoperative monitoring was carried out with f_R , an electrocardiogram (lead II), pulse oximetry (SpO₂), measurement of in- and expired gas concentrations, esophageal temperature and invasive blood pressure. Total anesthesia time was 330 minutes; total mass manipulation was 120 minutes and, for the removal of the vena cava invasion, the vessel was clamped for 14 minutes. After an abdominal opening, a liver neoformation was identified and, in adjunct, a hepatic lobectomy was performed.

The induction was smooth without significant hemodynamic alterations (Figure 2). The end-tidal isoflurane concentration was set between 0.9 and 1.2 % in order to maintain a stable anesthetic plane. During the procedure, the mean HR was 95 beats min⁻¹ (range 78-144) and arrhythmias were never recorded. The mass manipulation induced a mild increase in the mean HR (from 93.5 beats min⁻¹ before mass manipulation to 99.0 beats min⁻¹ during mass manipulation) and in mean MAP (from 71.8 mmHg before mass manipulation to 73.9 mmHg during mass manipulation). At the moment of the vena cava clamping, the MAP dropped to a mean of 61 mmHg (range 50-70 mmHg) and the mean HR increased to 121 beats min⁻¹ (range 95-144). The hypotension was dealt with by means of the administration of a bolus of Lactated Ringer's solution (10 ml kg⁻¹). At the end of the procedure, the isoflurane was discontinued and the dog was extubated five minutes later; the recovery was uneventful with the exception of mild bleeding from the subcutaneous tissues. Soon after extubation, the remifentanyl and dexmedetomidine infusions were discontinued. Postoperative analgesia was provided with lidocaine and fentanyl CRI until the following day and then with methadone (0,1 mg kg⁻¹) as needed. The histopathological evaluation revealed a diagnosis of adenocarcinoma.

The data were elaborated using Microsoft Excel (version 15.27, 2016; Microsoft corporation, WA, USA).

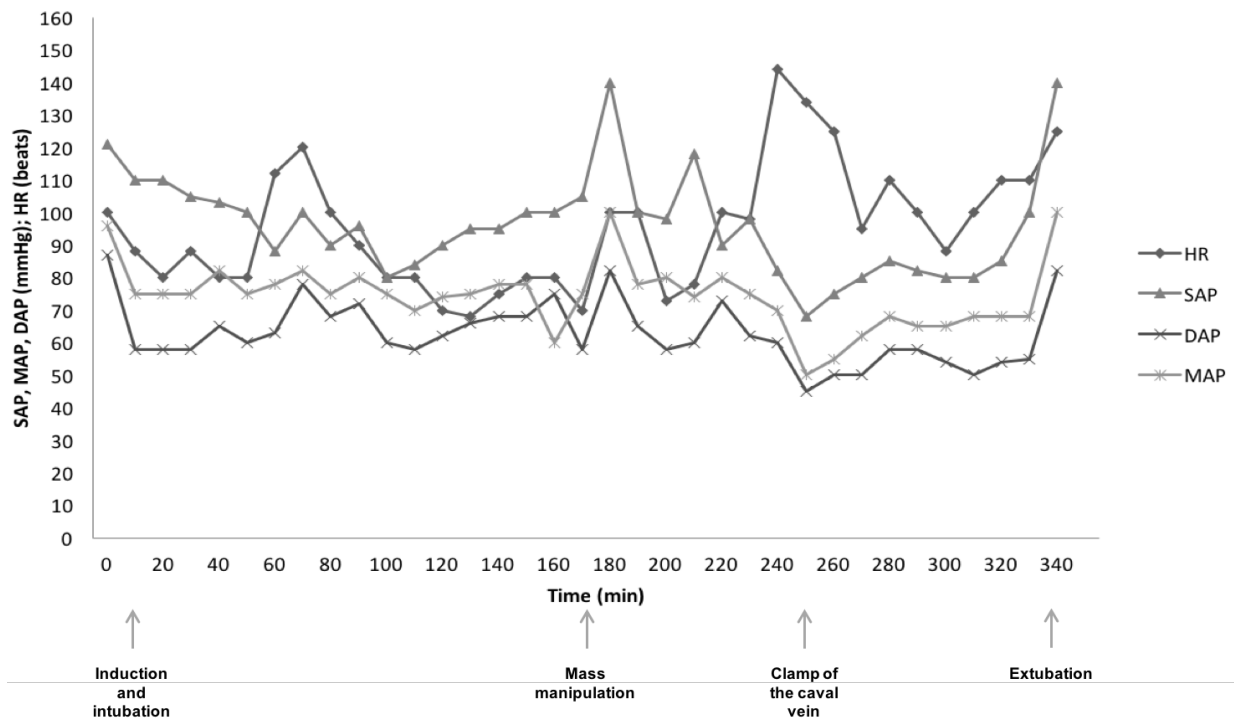


Figure 2- Cardiovascular parameters for CASE 2 recorded before sedation, at induction and intubation, during mass surgical manipulation (T170), during vena cava clamping (T240) and at the time of extubation (T340): Heart rate (HR), systolic arterial pressure (SAP), diastolic arterial pressure (DAP) mean arterial pressure (MAP). Dexmedetomidine ($0.0005 \text{ mg kg}^{-1}\text{h}^{-1}$) and remifentanyl ($0.0003 \text{ mg kg}^{-1}\text{min}^{-1}$) were administered by constant rate infusion throughout the anesthesia.

Discussion

In the two cases reported, dexmedetomidine along with remifentanyl provided satisfactory perioperative sedation for the two dogs with suspicion of PHEO and contributed to maintaining a stable intraoperative hemodynamic control with transient cardiovascular alterations recorded only during mass manipulation.

In both dogs histological and immunohistochemical evaluations diagnosed an adenocarcinoma. Adenocarcinoma of the adrenal cortex and PHEO may coexist (Galeotti and Marcato 2008). The diagnosis of PHEO is challenging: clinical signs, clinico-pathological results and data obtained with the diagnostic imaging are not helpful. The measurement of the catecholamines level in plasma or urine is more useful. At the microscopic evaluation the distinction between a PHEO from an adrenocortical tumor may be difficult and the specificity and sensitivity of the immunohistochemical markers in dogs have not been evaluated yet (Reusch 2015). In the first case described the dog had increased HR and blood pressure, the metanephrine levels were increased and the ACTH stimulation test was negative. The second dog included had increased Normetanephrine: creatinine ratio. In addition, in both dogs the mass manipulation induced transient increase in HR and blood pressure, as commonly described in case of pheochromocytoma.

During adrenalectomy for PHEO, the mortality and morbidity of patients are still high due to the cardiovascular instability which seems to be related to excessive catecholamine activity during mass manipulation by the surgeon (Gilson et al. 1994). The major life-threatening complications reported which are associated with PHEO ablation are hypertension, hypotension, arrhythmias and pulmonary edema. These can arise intraoperatively but also in the postoperative period (Herrera et al. 2008; Adams et al. 2015). Wide variations in blood pressure have commonly been described for this type of procedure. Sodium nitroprusside is the drug of choice to treat severe hypertension; it is a potent vasodilator with rapid onset and short duration of action. However, it can exacerbate the post resection hypotension which is commonly reported when catecholamine levels decrease as a consequence of

severe blood loss (James 1989). In the study of Gilson and colleagues (1994), four out of the six dogs included experienced hypotension which was treated with high volumes of crystalloid fluids. Only one dog required an infusion of dobutamine but remained hypotensive at recovery. Among arrhythmias, atrial premature contractions, ventricular premature contractions, ventricular tachycardia, supraventricular tachycardia and ventricular fibrillation have been recorded (Gilson et al. 1994; Herrera et al. 2008; Adams et al. 2015). Of the antiarrhythmic drugs, lidocaine, esmolol and magnesium sulfate (MgSO_4) have been used. Esmolol is a beta-antagonist with a rapid onset of action which is indicated for the treatment of sinus or supraventricular tachycardia (Adams et al. 2015). Magnesium sulfate blocks catecholamine release from adrenal medulla and peripheral nerve terminal, and also blocks the catecholamine receptors (Minami et al. 2002). It has been used in humans for pheochromocytoma ablation as a vasodilator and an antiarrhythmic drug (James 1989). Magnesium sulfate has also been used in combination with dexmedetomidine with the aim of decreasing the total amount of MgSO_4 , administered thereby limiting the risk of post resection hypotension (Bryskin and Weldon 2010). In that report, however, MgSO_4 and dexmedetomidine did not prevent an increase in HR and blood pressure during mass manipulation, and additional low doses of fentanyl, esmolol and nicardipine were needed (Bryskin and Weldon 2010).

In the present cases, the average HR and arterial blood pressure were within normal ranges for almost all the procedures. Only during surgical mass manipulation did the HR and MAP increase but these alterations were time-limited and did not need any additional treatment. In the second case described, the vena cava clamping induced a reduction in the venous return with subsequent transient hypotension responsive to an IV bolus of crystalloid fluids which resolved after the cessation of the clamping.

In a previous study describing the surgical treatment of pheochromocytoma in dogs, the following anesthetic protocols were used: acepromazine and atropine, atropine and oxymorphone, atropine, acepromazine and oxymorphone or oxymorphone alone (Gilson et al. 1994). However, in this type of procedure, anticholinergic and acepromazine can potentially aggravate the cardiovascular

status of the patient; atropine can aggravate arrhythmia, and the vasodilation induced by acepromazine could make the treatment of hypotension more difficult (Adams et al.2015).

In the present study, dexmedetomidine was administered in the perioperative period because of its anesthetic sparing effect, its analgesic properties and its modulating effect on catecholamine outflow. In dogs, a medium dose of dexmedetomidine administered by CRI ($0,0005 \text{ mg kg}^{-1} \text{ h}^{-1}$) reduced the minimum alveolar concentration (MAC) of isoflurane by 18% as compared to saline-treated dogs (Pascoe et al. 2006). The same dose of dexmedetomidine did not induce significant changes in HR and blood pressure as compared with a control group with the exception of an increase in diastolic blood pressure over baseline (Pascoe et al. 2006). In a more recent study, the same dose of dexmedetomidine ($0,0005 \text{ mg kg}^{-1} \text{ h}^{-1}$) administered over 180 by CRI in isoflurane-anesthetized dogs has been reported to decrease the HR and the cardiac index (CI) up to 17% (Pascoe 2015). In this latter study, the authors postulated that changes in the HR of less than 20% from baseline might be a good indicator of minor changes in the CI. In the present study, the CI was not evaluated and other drugs administered might have influenced the CI and the HR; in addition the blood pressure in our patients could have been influenced by the mass manipulation. Therefore, this evaluation would not be significant as regards the effects of dexmedetomidine on the CO and CI.

Remifentanil is an ultrashort synthetic pure mu opioid agonist. In dogs, remifentanil $0.0003 \text{ mg kg}^{-1} \text{ min}^{-1}$ has been proven to produce the maximal isoflurane sparing effect, and its prolonged infusion does not prevent a rapid recovery (Michelsen et al. 1996; Monteiro et al. 2010). In these studies, the use of dexmedetomidine in combination with remifentanil resulted in a lower fraction of expired isoflurane with respect to the MAC reported in dogs ($1.28 \pm 0.6\%$) by Steffey and Howland (1977). The anesthesia of the first dog was maintained with a fraction of expired isoflurane ranging from 1 to 1.2% and, for the second dog, the median fraction of expired isoflurane was 1%.

In human medicine, the perioperative administration of dexmedetomidine and remifentanil did not prevent the hemodynamic instability associated with surgical mass manipulation (Jung et al. 2012). In our patients the treatment with phenoxybenzamine, for at least two weeks as suggested for

humans in *The NANETS Consensus Guideline* (Chen et al. 2010), likely contributed to reducing perioperative complications.

In conclusion, dexmedetomidine infusion with remifentanyl provided satisfactory perioperative sedation and analgesia as well as acceptable intraoperative hemodynamic control for two canine patients with a suspicion of pheochromocytoma which received preoperative treatment with phenoxybenzamine. Additional studies are needed which would include a larger number of cases in order to better evaluate the feasibility and effectiveness of the present anesthetic protocol.

Alpha₂-agonists for the perioperative management of anesthesia in non-human primates undergoing craniotomy

In human medicine alpha₂-agonists have been described as safe and effective adjuncts to perioperative anesthetic protocols for patients submitted to neurosurgical procedures (Cormack et al. 2005; Peng et al 2014). In these patients the hemodynamic stability is pivotal. In the normal brain, cerebral blood flow (CBF) is maintained constant by autoregulation's mechanisms in front of alterations in systemic blood pressure within 50 and 150 mmHg. Hypertension can cause forced arteriolar vasodilation with subsequent blood-brain barrier alterations and cerebral edema formation. Hypotension, on the other side, with a MAP lower than 40 mmHg, could lead to a decreased CBF with subsequent cerebral ischemia and mental impairment (Lassen and Christensen 1976).

A meta-analysis investigating the application of dexmedetomidine in human patients undergoing intracranial surgery had shown that dexmedetomidine administration is associated with less perioperative intervention to maintain blood pressure and HR within normal ranges and with less perioperative general anaesthetics and opioids requirements (Peng et al. 2014). In a study on human patients undergoing craniotomy for tumor removal, dexmedetomidine (0.001 mg kg⁻¹ followed by a CRI of 0.0004 mg kg⁻¹h⁻¹) administered together with fentanyl CRI (0.001 mg kg⁻¹ h⁻¹) provided a significant higher decrease of intracranial pressure (ICP) compared to those patients that received fentanyl alone. In the same study, at the recovery, the Glasgow coma scale improved in patients receiving dexmedetomidine and deteriorated in those that did not receive the alpha₂-agonist; this difference was statistically significant (Soliman et al. 2011). Similarly, Kaushal and colleagues (2013) in their study on human patients with intracranial tumors, have reported that dexmedetomidine given before induction, reduced the HR by 10.48% and SAP by 16.45% upon intubation. In the

control group, instead, in which saline solution was administered before intubation, HR and SAP increased by 32% and 10.49 % respectively.

In animal models, dexmedetomidine has been proven to reduce the CBF (Ganjoo et al. 1998; Zornow et al. 1990; Zornow et al. 1992). In healthy dogs, anaesthetized with halothane or isoflurane, dexmedetomidine administration reduced the CBF by 30-45%, despite a concurrent increase in systemic arterial pressure (mean value 180 mmHg) above limits of autoregulation, and without causing significant changes in cerebral metabolic rate for oxygen (Zornow et al. 1990; Karlsson et al. 1990). In rabbits, dexmedetomidine either reduced ICP by 30% or prevented a significant increase in ICP despite an increased systemic arterial pressure when administered at 0.08 or 0.32 mg kg⁻¹ respectively) (Zornow et al. 1992). In addition in the same study authors found that also in presence of cerebral cryogenic lesions, dexmedetomidine (0.32 mg kg⁻¹) did not increase ICP if compared with the placebo treated group (Zornow et al. 1992).

Alpha₂-adrenoceptors have been identified in cerebral microvessels in humans and animal models (Harik et al 1981; Ferrari-DiLeo and Potter 1985). Dexmedetomidine, through the activation of post-synaptic alpha₂-adrenoceptors in the cerebral vessels, increases cerebral vascular resistance preventing significant alteration of the CBF (Zornow et al. 1990; Prielipp et al. 2002). In fact, in dogs dexmedetomidine decreased significantly the vasodilation induced on cerebral vessels by halogenated anesthetics. This effect was prevalent on small or large arterioles and on small venules and was independent on the dose of dexmedetomidine administered (Ohata et al. 1999). The action of these drugs on alpha₂-adrenoceptors in the *locus coeruleus* also decreased the regional blood flow in that area.

Other advantages provided by alpha₂-agonists may be beneficial during anesthesia for neurosurgical procedures. In facts, through their sympatholytic effects, alpha₂-agonists, reduces the catecholamine release and therefore prevents the increase in systemic arterial pressure associated with the surgical stimulation or with the intubation (Kaushal et al. 2013; Peng et al. 2014). In addition,

alpha₂-agonists through their anesthetic sparing effect reduce the amount of general anesthetic, contributing in the maintenance of a stable anesthetic plane (Ewing et al. 1993; Pascoe et al. 2006).

Non-human primates are widely used as experimental animal models for neurophysiological study because of their resembles to humans. Besides their adaptability in laboratory facility, sedation is often required to perform also minimally invasive procedures to facilitate handling and to minimize risks for the operator: they can bite and they are potentially a reservoir of zoonotic diseases.

Ketamine (5-20 mg kg⁻¹) has been used as a sole agent in primates to obtain sedation. However, alone it does not provide muscle relaxation but tonic-clonic movements, muscle damage upon injection and alteration in food intake have been reported after its administration (Popilskis et al. 2008). Consecutive administration of ketamine (10 mg kg⁻¹) resulted in a cumulative effect, in muscular damage and in increased liver enzymes (Lugo-roman et al. 2010). The combination of ketamine with alpha₂-agonists has been described in non-human primates by several authors as an effective alternative to provide deeper immobilization compared with ketamine alone; in addition the effects of alpha₂-agonists can be reversed with the administration of atipamezole (Sun et al. 2003; Lugo-roman et al. 2010). The concomitant administration of medetomidine (0.05 mg kg⁻¹) allowed a reduction in the dose of ketamine (2 mg kg⁻¹) necessary to obtain an effective anesthesia. This combination resulted in a lower increase in liver and muscular enzymes compared with ketamine administered alone (Lugo-roman et al. 2010). Sun and colleagues (2003) have compared ketamine (10 mg kg⁻¹) alone and in combination with medetomidine (K 3.0 mg kg⁻¹ and M 0.15) in *Macaca*. They have described that ketamine together with medetomidine provided a longer lasting (71.22 ± 7.6 minutes vs 27.81 ± 2.6 minutes) and more profound sedation if compared with ketamine as a sole sedative drug (Sun et al. 2003). In the same study authors administered a similar ratio of the same anesthetic combination or ketamine alone IM to rats with the aim to evaluate their effects on tissue. They observed that inflammation and necrosis were significantly more severe when ketamine was administered alone (Sun et al. 2003).

In another study similar dosages of medetomidine and ketamine (M 0.15 mg kg⁻¹ and K 3 mg kg⁻¹) provided considerable benefits compared with ketamine alone: a greater muscle relaxation and deeper sedation with an onset of 3.1 ± 0.4 minutes (Lee et al. 2010). Animals receiving this anesthetic mixture presented a lower HR, than those treated with ketamine, without significant differences in blood pressure, in PaCO₂ and in PaO₂ (Lee et al. 2010). The administration of atipamezole (0.1-0.2 mg kg⁻¹) resulted in the reverse of the sedative effect with a recovery time of 8.7 ± 1.8 minutes (Lee et al. 2010; Ochi et al. 2014). Otherwise, medetomidine administered alone did not provide an effective sedation even for minimally-invasive procedures. Medetomidine 0.05, 0.1, 0.15 and 0.2 mg kg⁻¹ administered IV in Rhesus macaques induced a deep sedation but characterized by sudden and aggressive arousal (Capuano et al. 1999).

To the author knowledge there are no study describing the administration of an alpha₂ agonist by CRI in non-human primates undergoing craniotomy.

In the paper *Constant-Rate Infusion of Dexmedetomidine to Manage Thiopental Anesthesia during Intracranial Surgery in Cynomolgus Macaques (Macaca fascicularis)* the perioperative management of *Macaca fascicularis* undergoing craniotomy for neurophysiological studies with dexmedetomidine and thiopental administered by CRI for maintenance of general anaesthesia has been described. The paper has been published in the *Journal of the American Association for Laboratory Animal Science* (vol 55 no 6, November 2016) and has been reported here with the consensus of the Editor.

Constant-Rate Infusion of Dexmedetomidine to Manage Thiopental Anesthesia during Intracranial Surgery in Cynomolgus Macaques (*Macaca fascicularis*)

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Macaques (*Macaca* spp.) are often used as animal models in biomedical research involving a neurosurgical approach. The development of new anesthetic techniques is pivotal for these studies. Studies in human anesthesia for intracranial surgery have shown that dexmedetomidine infusion reduces the incidence of cardiocirculatory complications in the perioperative period, reduces the need for supplemental analgesia, and provides an analgesic effect analogous to that of remifentanyl. Data regarding the anesthetic effects of dexmedetomidine infusion in NHP including *Macaca* spp. are currently unavailable. The study population comprised 5 healthy cynomolgus macaques (*Macaca fascicularis*) that underwent intracranial surgery. On the day of surgery, the subjects were sedated with intramuscular ketamine (8 mg/kg) and dexmedetomidine (0.02 mg/kg). Anesthesia was induced with thiopental (3 mg/kg IV) and maintained by using constant-rate infusion of thiopental (3 mg/kg/h); analgesia was provided by constant-rate infusion of dexmedetomidine (0.012 mg/kg/h). Atipamezole (0.1 mg/kg IM) was administered at the end of the surgical procedure. The median heart rate increased after sedation, reaching its highest level at 60 min (91.0 ± 6.9 bpm); the highest systolic blood pressure (119.6 ± 10.5 mm Hg) occurred at 75 min. No animal experienced respiratory arrest, and all recovered within 6 min after atipamezole administration. In cynomolgus macaques, dexmedetomidine constant-rate infusion provided adequate analgesia and stable hemodynamic control. Using dexmedetomidine as an adjunct to thiopental-maintained anesthesia may be advantageous in healthy NHP undergoing intracranial surgery.

Cynomolgus monkeys (*Macaca fascicularis*) are often used as animal models in behavioral and biomedical research due to their phylogenetic affinity to humans. Chemical restraint and anesthesia are often required for the care of these species or for the purposes of the research in which they are involved.

The α_2 -adrenoreceptor agonists are a class of drugs widely used to produce sedation, anxiolysis, analgesia, muscle relaxation, and a sparing effect of injectable and inhalant anesthetics in different species.¹⁶ Moreover, they play a key role in NHP pharmacologic immobilization.^{4,17,20,21} Various studies have demonstrated that medetomidine, a highly selective α_2 agonist, produced a reliable loss of consciousness in rhesus monkeys when used alone or in association with ketamine, midazolam, or fentanyl.²⁰

Dexmedetomidine is the dextroisomer of medetomidine formulation and, at half the dose of medetomidine, it produces a similar effect.^{2,8} Studies in human anesthesia for intracranial surgery have shown that dexmedetomidine infusion decreases the incidence of cardiocirculatory complications in the perioperative period, reduces the requirements of supplemental analgesia, and has an analogous analgesic effect to remifentanyl.¹² In dogs, dexmedetomidine infusion has produced a marked reduction in cerebral blood flow in healthy animals.²² In rabbits, in the presence or absence of cryogenic lesions, dexmedetomidine infusion had minimal effects on intracranial pressure.²³

Various studies involving dogs have investigated the effect of dexmedetomidine at constant-rate infusion during isoflurane anesthesia.^{6,10,11} At low doses, dexmedetomidine infusion (from 0.1 to 0.5 $\mu\text{g/kg/h}$) yielded acceptable cardiopulmonary changes, whereas a rate infusion of 3 $\mu\text{g/kg/h}$ produced significant cardiorespiratory depression.^{10,11} Only 2 studies have investigated the effects of a single dexmedetomidine bolus in NHP for chemical restraint, and no data have been reported regarding constant-rate infusion in this species.^{15,19}

We hypothesized that dexmedetomidine infusion in *Macaca fascicularis* undergoing intracranial surgery would provide analgesia, cardiocirculatory stability and a sparing effect of the thiopental dose necessary for maintaining a satisfactory plane of anesthesia. The aim of this study was to evaluate cardiocirculatory, respiratory, and anesthetic variables during dexmedetomidine infusion and thiopental general anesthesia in *Macaca* undergoing intracranial surgery.

Materials and Methods

Five male cynomolgus macaques (*Macaca fascicularis*; age, 5 to 12 y; weight, 3 to 11 kg) were involved in the study. Each primate underwent intracranial surgery for the purpose of anatomic and physiologic research that was approved by the Ethical-Scientific Committee of the University of Bologna and authorized by National Competent Authorities in accordance with Legislative Decree number 116/92 (enacting Council Directive 86/609/ECC). At their arrival, the macaques were certified free from herpes B virus, SIV, simian retrovirus, and

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simian T lymphotropic virus. The animals had never been used previously in an experiment.

The macaques were housed individually in appropriately sized cages, and environmental enrichment was provided in accordance with standard operating procedures. Twelve hours before the surgical procedure, food was withheld but water was available free choice. Two of the 5 animals were anesthetized to implant 2 metal plates on the skullcap for electrophysiologic studies; the remaining 3 macaques underwent craniotomy to inoculate anterograde tracers in the white matter of the brain for anatomic study.

On the day of the surgery, the macaques received ketamine (8 mg/kg IM; Ketavet, Intervet Productions, Latina, Italy) and dexmedetomidine (0.02 mg/kg IM; Dexdomitor, Elanco Animal Health, Hampshire, United Kingdom) in the gluteal muscles. The animals were immobilized for injection by using the retractable rear wall of the cage. Sedation time was defined as the time between the intramuscular injection and the moment when the animal was laterally recumbent on the floor of the cage (time 0). As soon as the macaques became recumbent, the clinical sedation score was determined in regard to response to firm pressure applied to the digits (grip reflex) and response to catheterization of the femoral vein (scored as 'yes' or 'no'). The macaques were removed from the cages and carried to the operating theater. The left saphenous vein was catheterized by using a 22-gauge angiocatheter (Delta Med, Milan, Italy), and all monitoring devices were attached before the induction of anesthesia. A continuous lead II ECG was obtained by using a multiparameter monitor (Cardioline, Milan, Italy), and the pulse rate and arterial oxygen saturation were monitored by pulse oximetry (probe placed on the first anterior digit). The respiratory rate was determined by visually monitoring thoracic expansion over 1 min. Body temperature was measured by using a rectal temperature probe. Blood pressure was monitored by using a Doppler device (Minidrop ES-100 VX, Hadeco, Tokyo, Japan).³ In brief, the Doppler probe was placed over the common digital branch of the metatarsal artery on the dorsal aspect of the foot; the width of the cuff was approximately 40% of the limb circumference, as described previously.¹⁸ A no. 3 cuff was used for each animal. The hair was clipped before placing the probe. Ultrasound transmission gel (Aquasonic 100, Parker Laboratories, Fairfield, NJ) was placed between the probe and the skin to improve contact, and the volume of the Doppler machine was adjusted to obtain a clearly audible signal. Five measurements were performed; the highest and the lowest values were excluded, and the mean of the remaining values was calculated. All of these values and the presence of the grip reflex were recorded at 5-min intervals for the duration of the entire procedure. A forced-air warming blanket (Bair Hugger, 3M, Berkshire, United Kingdom) was used to maintain physiologic body temperature. After the first monitoring was completed, a bolus of thiopental (3 mg/kg IV; Pentothal, Intervet Productions) was administered, and infusion (3 mg/kg/h) was started by using an infusion pump (Angle syringe pump, CRIMO, Forlì-Cesena, Italy). Dexmedetomidine infusion (0.012 mg/kg/h) and lactated Ringer solution were administered (5 mL/kg/h) by using a peristaltic pump (B-Braun, Melsungen, Germany). Oxygen (2 L/min) was provided through a human pediatric facemask. The macaque's head was placed into the stereotaxis instrument, with the animal in the sphinx position. The animal was fixed to the stereotaxic frame by means of tapered ear bars which fit into the external auditory meatus. Adjustable infraorbital clamps, and a vertically adjustable palate bar prevented rotation of the skull. The stereotaxic apparatus

limited the possibility of endotracheal intubation because of the encumbrance of the palate bar.

If a grip reflex or responses to stimuli were present, a bolus of thiopental (2 mg/kg IV) was administered. All these events were recorded. In the case of hypotension, defined as systolic blood pressure of less than 80 mm Hg, dopamine infusion from 5 to 10 µg/kg/min was administered IV. At the end of the procedures, atipamezole (0.1 mg/kg IM; Sedastop, Esteve, Oudewater, Netherlands) was administered. Time of recovery was defined as the time from the end of the procedure and cessation of the thiopental until the animal was able to sit up in the cage. To record any possible side effects, such as dysphoria and vomiting, the anesthetists observed the macaques until they were fully awake.

Statistical analysis. All data are reported as mean \pm 1 SD. The baseline heart rate, respiratory rate, and systolic blood pressure (measured after sedation with dexmedetomidine and ketamine) were compared between time points by using the Wilcoxon test (paired samples) (MedCalc 6.3, MedCalc Software, Ostend, Belgium). A *P* value of less than 0.05 was considered statistically significant.

Results

The sedation was smooth, and in 3 to 5 min, all of the cynomolgus macaques were lateral recumbent and unresponsive to manipulation. At the evaluation of the sedation score, the palpebral reflex was depressed, the menace and grip reflexes were lost, and muscle relaxation was good. No animal reacted during the positioning of the venous catheter.

The procedure time was 204 \pm 121.2 min (mean \pm 1 SD), depending on the type of surgery. The cardiovascular parameters are summarized in Figure 1. The heart rate increased after sedation increasing to 91 \pm 7 bpm at 60 min. During the procedure, 2 macaques experienced sinus bradyarrhythmia consisting of a regular variation of rate associated with respiration. No other rhythm abnormalities were detected. The lowest systolic blood pressure measured in each animal was the baseline value (93.8 \pm 5.7 mm Hg). During surgical manipulation, the highest blood pressure was measured at 75 min (119.6 \pm 10.5 mm Hg). The mean arterial oxygen saturation according to pulse oximetry at the first measurement point was 97% and was maintained throughout the surgical period. No respiratory arrest occurred; the respiratory rate ranged from 18 to 24 breaths per minute. During the surgical period, the rectal temperature remained stable at approximately 36.5 °C with the aid of a forced-air warming blanket. Neither heart rate, blood pressure, mean arterial oxygen saturation, nor rectal temperature differed between any time points.

Thiopental was maintained through constant-rate infusion of 3 mg/kg/h in most of the animals. Only one macaque (at 120 min after induction) showed a marked grip reflex and response to stimulus (gross movement of the legs) in the absence of changes in cardiorespiratory parameters. In this case, a thiopental bolus (2 mg/kg IV) was administered, and thiopental infusion rate was increased to a maximum of 8 mg/kg/h until the disappearance of the grip reflex. In the remaining 4 monkeys, when there was an unexpected positive response to the grip reflex, a thiopental bolus (2 mg/kg IV; 2 times for 3 monkeys) was administered, however we did not observe hemodynamic or other gross movement associated with any surgical stimulus (incision of the skin, craniotomy, manipulation of the meninges). The average recovery time from anesthesia after atipamezole administration was 6.0 min (range, 4.5 to 7.0 min). All of the macaques awoke smoothly, without dysphoria or side effects.

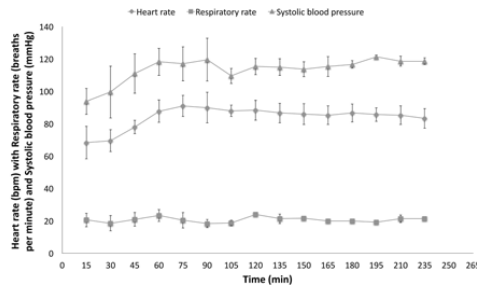


Figure 1. Cardiovascular and respiratory parameters recorded from sedation (time 0) to the end of a neurosurgical procedure in cynomolgus macaques.

Discussion

The present study confirmed that dexmedetomidine infusion in cynomolgus macaques undergoing intracranial surgery provided sufficient analgesia to maintain an adequate plane of anesthesia. The combination of ketamine and dexmedetomidine produced effective immobilization with an onset of less than 5 min in all 5 macaques treated.

In NHP, thiopental constant-rate infusion (15 to 17 mg/kg/h), used to maintain general anesthesia, has been reported to provide satisfactory chemical restraint.¹³ In the present study, the thiopental rate infusion was initially set at 3 mg/kg/h. Only 1 of the 5 macaques demonstrated a positive grip reflex and gross movement in response to surgical stimulus; administration of a thiopental bolus (2 mg/kg IV) was insufficient to achieve a stable anesthetic plane, so we increased the thiopental infusion rate to 8 mg/kg/h. This dosage is still lower than that previously reported in NHP.¹³ The choice of the initial thiopental infusion rate was justified because, in our experience, previously reported infusion rates¹³ associated with dexmedetomidine led to excessively deep sedation. In fact, the contemporary use of dexmedetomidine, as reported in small animal anesthesiology studies, decreased the intraoperative requirement of the drug used for the maintenance of general anesthesia.^{8,10} Comparison with a control group of macaques anesthetized with thiopental infusion only might be useful in determining the dosages necessary to maintain anesthesia in the absence of dexmedetomidine infusion and in assessing the sparing effect of the sedative drug. However, the approved protocol did not include a control group and, therefore, the dosages were extrapolated from a previously published study.¹³

Dexmedetomidine produces bradycardia and initial hypertension, as do other α_2 -adrenoreceptor agonists.^{14,15} In the present study, assessment of pretreatment baseline values for heart rate and blood pressure was infeasible due to risk to the handler and the need to minimize stress to the subjects. The normal resting heart rate of cynomolgus macaques is between 100 to 200 bpm.⁹ After sedation, the first recorded heart rate was 68.4 ± 7.4 bpm, whereas the mean heart rate ranged from 69.6 to 94.0 bpm during the procedure and therefore can be defined as bradycardia when compared with the normal values previously reported.⁹ The bradycardia was due to the action of the dexmedetomidine on central and peripheral α_2 adrenoreceptors, as described previously for NHP and in small animals.^{4,16} The bradycardia was accompanied by a respiratory sinus arrhythmia, which might have been related to the action of dexmedetomidine on central α_2 -adrenoreceptor agonists, with a reduction of sympathetic outflow.¹⁶ In the event of life-threatening bradycardia or arrhythmias, atipamezole should

be administered to reverse the cardiocirculatory side effects of dexmedetomidine.⁷ Another potential explanation for the bradycardia is an increase in intracranial pressure consequent to the surgical procedure, as has been reported in human patients.¹ However, the bradycardia in our macaques began before the intracranial surgical procedure was initiated.

Throughout the intracranial surgery, the systolic blood pressure remained stable, at 93.8 ± 5.7 to 119.6 ± 10.5 mm Hg, in all 5 macaques. The lowest value was measured at the first time point. This finding seems to be in contrast to the biphasic arterial pressure (initial hypertension followed by hypotension) due to dexmedetomidine injection in dogs.¹⁴ The effect in our study might be explained by a decrease in central sympathetic tone that overcame the peripheral cardiovascular effects.¹⁶ None of our macaques experienced hypotension, and none received dopamine infusion.

In the current study, the respiratory rate showed an initial decrease, but the arterial oxygen saturation according to pulse oximetry remained within the normal range throughout surgery. Pulse oximetry is widely considered the optimal method for noninvasive continuous monitoring of the oxygen saturation of arterial hemoglobin; however to overcome the limitations of this technology, a blood-gas evaluation of the oxygen partial pressure should be performed.⁵ In the present study, the pulse oximetry did not drop below 97%, and the wave tracing on the monitor remained stable throughout surgery; we therefore considered blood-gas analysis to be unnecessary. However the anesthetist should evaluate capnography and end-tidal CO_2 to confirm adequate ventilation. A criticism regarding the anesthesia management of these 5 macaques was the inability to intubate them due to the position of their heads in the stereotaxic apparatus and the lack of blood-gas analysis to control the pCO_2 . During intracranial surgery, monitoring of end-tidal CO_2 or pCO_2 enables detection of hypercapnia. In fact, hypercapnia decreases the blood pH, which induces vasodilation in the brain and consequently increases cerebral blood flow and intracranial pressure.

In small animals, α_2 -adrenoreceptor agonists typically induce vomiting due to their direct action on the chemoreceptor trigger zone.¹⁶ Similarly, in NHP, vomiting occurs during the perioperative period; therefore, endotracheal intubation is recommended to avoid aspiration pneumonia, which is a risk even in food-fasted animals. However, endotracheal intubation was not possible in the current study, due to interference from the palate bars of the stereotaxic instrument. Regardless, none of the 5 macaques vomited during the perioperative period, nor were secretions or foreign materials detected in the mouth at the end of the procedure.

Increased intracranial pressure is often a complication of intracranial surgery, and anesthetic drugs can exacerbate the hemodynamic changes in brain perfusion. We did not evaluate intracranial pressure directly, but the surgeons did not report any increase in bleeding or tissue edema during the procedure, and the anesthetist did not note any dysphoria during recovery. Dexmedetomidine reduces cerebral blood flow through α_2 -adrenoreceptor-mediated vasoconstriction, and atipamezole administration allowed rapid recovery from anesthesia, as has previously been reported after the administration of medetomidine in NHP.^{17,21}

The current study confirmed that the use of dexmedetomidine infusion in healthy cynomolgus macaques undergoing intracranial surgery provided stable sedation and adequate analgesia for neurosurgical procedures. Furthermore, our findings demonstrated that dexmedetomidine administered

through constant-rate infusion to cynomolgus macaques as an adjunct to thiopental-maintained anesthesia provided good cardiovascular stability and reduced the dose of thiopental necessary for maintaining adequate sedation for neurosurgical procedures, compared with dosages previously reported.¹³ During the perioperative period, the macaques' hemodynamic parameters remained in acceptable clinical ranges in the absence of cerebral bleeding or edema. Additional studies to determine the effects of dexmedetomidine on the cerebral blood flow and associated changes in the intracranial blood pressure of NHP are warranted.

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Discussion and conclusion

In the present work, new applications of α_2 -agonists in veterinary medicine have been described.

α_2 -agonists are widely used in veterinary anesthesia because of their sedative, analgesic, myorelaxant and anesthetic sparing effect properties. In addition, because of the widespread distribution of the α -adrenoceptors their application can be extended to different clinical application other than the anesthetic one. The administration of α_2 -agonists to obtain ejaculation and semen collection in different species has been reviewed in the present work. In cats the Ur.Ca.P.I. protocol was first described by Zambelli and colleagues and is based on the administration of high dose medetomidine (0.13 mg kg^{-1}) for semen collection. When lower doses of medetomidine (0.05 mg kg^{-1}) were administered to cats low quality semen samples were collected compared with trials in which higher doses were used (0.13 mg kg^{-1}). Further studies are needed to verify the expression of α -adrenoceptors in the genital tract of male cats and the dose dependent affinity of medetomidine for these receptors. This is the topic of ongoing research (unpublished results).

However, studies have described that even when α_2 -adrenoceptor agonists are administered at clinical doses in healthy patients they can induce bradycardia, arrhythmia, decreased CO and blood pressure alterations (England and Clarke 1996; Pypendop and Verstegen 1998; Ko et al. 2001; Lamont et al. 2001; Carter et al. 2010). These cardiovascular side effects cause concerns among veterinary practitioners and commonly limit their administration to healthy animals. We have therefore investigated the hemodynamic effects of high dose medetomidine (0.13 mg kg^{-1}) administered in healthy cats. In our study significant hemodynamic alterations after medetomidine administration were observed. In particular we have observed a significant decrease in HR and in CO and significant alteration in diastolic and systolic functions. However, similar echocardiographic

alterations had been previously reported even after lower doses of medetomidine (0.02 mg kg^{-1}) (Lamont et al. 2001). We can therefore conclude that the hemodynamic effects induced by medetomidine are not dose-dependent.

Besides these significant cardiovascular changes, no clinically significant alterations were detected at the recovery; however, in this study healthy cats were included and we can't foresee if high doses medetomidine don't significantly impair the hemodynamic functions in patients with cardiovascular diseases. Therefore, when possible, a complete physical examination and a cardiovascular evaluation should be performed before the administration of α_2 -agonists, especially if high doses are used.

Besides the undesirable cardiovascular effects, low doses of α_2 -agonists, by means of their action on α -adrenoceptors and imidazole receptors, induce cardiovascular, respiratory and metabolic alterations that may offer advantages for specific clinical conditions. In fact, in human medicine, α_2 -agonists have been described as effective adjuncts to improve the hemodynamic stability in patients undergoing specific procedures like pheochromocytoma ablation or neurosurgical procedures. In these patients the management of the cardiovascular stability is challenging.

In particular, during pheochromocytoma ablation, cardiovascular complications are common and are due to the catecholamine release during mass manipulation. Dexmedetomidine administration in patients with pheochromocytoma has been described as an effective adjunct to reduce the catecholamines' levels and to improve the cardiovascular stability (Wong and Cheung 2004; Shumann and Hudcova 2010; Khetarpal et al. 2014; Singh and Singh 2014).

Also in patients undergoing craniotomy the hemodynamic stability is pivotal. The cerebral blood flow in healthy patients is maintained constant despite narrow variations in arterial blood pressure but outside these ranges, CBF changes proportionally with the blood pressure. Dexmedetomidine, through vasoconstriction on cerebral microvessels prevented significant alterations in CBF despite concomitant variations in systemic blood pressure above limits of autoregulation (Zornow et al. 1990; Karlsson et al. 1990).

In the present work, preliminary results describing the use of dexmedetomidine for the anesthetic management of veterinary patients undergoing pheochromocytoma ablation and craniotomy have been reported in dogs and cynomolgus monkeys respectively. In both cases, dexmedetomidine was administered pre-operatively and intraoperatively by CRI. We did not observe any significant cardiovascular and neurological complications commonly associated with these procedures (Lassen and Christensen 1976; Gilson et al. 1994) In these studies, dexmedetomidine throughout its anesthetic and analgesic sparing effect reduced the dose of inhalant or injectable anesthetic agents necessary to maintain a stable anesthetic plane and this effect may have contributed in the maintenance of the cardiovascular stability. In addition, the decrease of the sympathetic tone and the inhibition of the catecholamine release provided by dexmedetomidine is beneficial in these patients. In fact, the tracheal intubation or the surgical stimulation may increase the catecholamine release with subsequent hypertension which can be detrimental in these situations.

There are several limits of these studies. First of all, only few animals were included: two dogs and five macaca respectively. In addition, in both studies only the non-invasive blood pressure was evaluated and in macaca neither the EtCO₂ or the PaCO₂ and the ICP or the CBF were assessed intraoperatively as previously described. Further studies concerning the use of alpha₂-agonists for the anesthetic management of veterinary patients undergoing pheochromocytoma ablation or craniotomy are needed, including more animals and using a complete cardiovascular monitoring. Moreover, the feasibility of the same protocols in different species should be evaluate. However, in the studies reported here, new application of alpha₂-agonists in veterinary patients have been described for the first time and these preliminary results support the use of dexmedetomidine as an anesthetic adjunct for the intraoperative management of canine patients undergoing pheochromocytoma ablation or for cynomolgus monkeys undergoing craniotomy. Due to the potentially significant hemodynamic changes induced by alpha₂ agonists, when possible, a complete clinical examination should be performed before their administration, especially if high doses of alpha₂-agonists are administered.

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