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Understanding the physiological basis for managing anaesthetic related cardiopulmonary side-effects in wildlife

Buss, Peter Erik

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UNDERSTANDING THE PHYSIOLOGICAL BASIS FOR MANAGING ANAESTHETIC RELATED CARDIOPULMONARY SIDE-EFFECTS IN WILDLIFE

Peter Erik Buss

A thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of
Doctor of Philosophy.

Johannesburg, South Africa, 2017

DECLARATION

This thesis is submitted in the optional format, approved by the Faculty, of published work with encompassing introduction and discussion.

I, Peter Buss, declare that the work contained in this thesis is my own, unless otherwise acknowledged. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. This work has not been submitted before for any degree or examination at any other University.



Dr Peter Buss

Signed on the 4th day of July, 2017 in Skukuza

ABSTRACT

Immobilization of white rhinoceros (*Ceratotherium simum*) is a fundamental procedure used in the conservation of this threatened megaherbivore and allows for the capture, translocation and treatment of individuals. Immobilization also allows scientific investigation, which facilitates protection of this species. The potent opioids, including etorphine, are the only class of drugs which allow for a rapid and reversible immobilization, which is essential in the capture of rhinoceros. However, immobilization is associated with changes in respiratory and cardiovascular function which can result in high morbidity and mortalities. I therefore investigated the cardiorespiratory pathophysiological effects of etorphine and azaperone; pharmacological agents most often used in rhinoceros immobilization, and examined the effectiveness of butorphanol, a mixed agonist-antagonist opioid, in limiting these adverse effects. Reducing morbidity and mortality risks through an increased understanding and moderation of drug-induced cardiorespiratory changes in immobilized rhinoceros will contribute to future successes in managing this species.

In my first study, ten healthy captive white rhinoceros including four males and six females ranging in age from 3.5 to 15 yr were immobilized for a total of 13 procedures with etorphine plus azaperone, and administered butorphanol intravenously immediately after initial blood collection and physiological assessment. Respiratory and cardiovascular parameters, body temperature and arterial blood gases were monitored for 100 min. The results confirmed that severe hypoxaemia, hypercapnia, tachycardia and an increased alveolar-arterial (A-a) oxygen gradient occur in immobilized rhinoceros. Giving butorphanol appeared to decrease heart rate, increase arterial oxygen tension, and decrease the A-a gradient and respiratory rate. However as the study was observational, it could not be confirmed that these changes were caused by butorphanol. Despite the initial improvements in blood oxygen levels, the rhinoceros remained severely hypoxaemic and hypercapnic for the remainder of the procedure.

To further investigate the cardiorespiratory effects of butorphanol in immobilized rhinoceros, a randomised cross-over study design was used. Six healthy sub-adult male white rhinoceros were subjected to four drug interventions: 1) etorphine intramuscularly; 2) etorphine plus azaperone intramuscularly; 3) etorphine intramuscularly and post-induction butorphanol intravenously; and 4) etorphine plus azaperone intramuscularly, and post-induction butorphanol intravenously. The results from this study demonstrated that hypoxaemia and hypercapnia in etorphine-immobilized rhinoceros were not predominantly a result of a decrease in respiratory minute volume, as has been proposed in previous studies. Rather, an increase in metabolic oxygen consumption and carbon dioxide production, associated with muscle tremors, is suggested as the primary cause. In addition, a high alveolar-arterial oxygen gradient may have contributed to hypoxaemia and possibly also hypercapnia in immobilized rhinoceros. Although, decreased minute ventilation was not the fundamental cause of hypoxaemia and hypercapnia, low arterial oxygen partial pressure (PaO_2) and high arterial carbon dioxide partial pressure ($PaCO_2$) did not stimulate ventilation, probably as a consequence of opioid-induced central and peripheral chemoreceptor inhibition. Butorphanol administered post-induction in etorphine-immobilized rhinoceros resulted in a moderate improvement in blood gases, although hypoxaemia and hypercapnia persisted. My results support the idea that improvements in PaO_2 and $PaCO_2$ after butorphanol administration resulted from reduced muscle tremors, metabolic oxygen consumption and carbon dioxide production rather than improved minute ventilation.

Cardiovascular changes in etorphine-immobilized rhinoceros included hypertension and tachycardia. The inclusion of azaperone with etorphine in the immobilizing drug combination reduced blood pressure to below normotensive values; however, heart rate remained elevated. The administration of butorphanol was followed by a reduction in heart rate with no clinical effect on blood pressures in etorphine-immobilized rhinoceros. Similarly, butorphanol did not change blood pressure but reduced tachycardia in individuals immobilized with etorphine plus azaperone.

In summary, butorphanol administration reduced hypoxaemia and hypercapnia in immobilized white rhinoceros as a result of decreased muscle tremors and oxygen consumption. Reduced oxygen consumption may mitigate hypoxic and hypercapnic mortality risks associated with immobilization, especially in rhinoceros compromised due to old age, nutritional stress or disease. My findings indicate that butorphanol administration allows rhinoceros to be immobilized for extended periods, which facilitates clinical procedures in injured individuals or managing orphaned calves. The reduction in tachycardia suggests that butorphanol may have a myocardial oxygen-sparing effect and may lower the risk of an adverse outcome associated with immobilization.

My recommendations for the immobilization of white rhinoceros to reduce the morbidity and mortality risks associated with etorphine-induced respiratory and cardiovascular changes include azaperone administration in combination with the potent opioid. The inclusion of azaperone reduces hypertension in etorphine-immobilized rhinoceros. However, I suggest that lower azaperone doses be considered compared to those used in my studies to moderate the decrease in blood pressure and possible complications associated with reduced tissue perfusion. Butorphanol should be administered intravenously to etorphine plus azaperone-immobilized-rhinoceros as soon as possible after induction to limit increased opioid-induced metabolic effects and improve hypoxaemia and hypercapnia. The administration of butorphanol also has the advantage of reducing tachycardia with a potential myocardial oxygen sparing effect in immobilized-rhinoceros. Butorphanol should be administered in repeated doses (5 to 10 x etorphine dose in mg) until limb muscle tremoring is reduced and possibly halted, and an elevated heart rate slows to less than 100 beats per minute or slower.

Future research should focus on improving alveolar gas exchange and reducing the sympathomimetic and hypermetabolic effects of the potent opioids in immobilized-rhinoceros, and not only just improving ventilation and blood pressure.

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I was responsible for developing and implementing the study idea. I performed the experimental procedures, collected, analyzed and interpreted the data, and wrote the first draft of the manuscript. Francisco Olea-Popelka assisted by Laura Martin, assisted with statistical data analysis and contributed to writing the manuscript. Leith Meyer, my supervisor, helped with interpretation of the data, and edited the manuscript. Jenny Hofmeyr, Nomkosi Mathebula, Marius Kruger and Angela Brüns assisted with experimental work, data collection and edited the manuscript. Michele Miller, my co-supervisor, supported the study idea development and implementation, assisted with data collection and interpretation, and edited the manuscript.

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The idea of the study arose from discussions with my supervisors, Leith Meyer and Andrea Fuller. I prepared the equipment, performed the experimental procedures, collected, analyzed and interpreted the data, and wrote the first draft of the manuscript. Francisco Olea-Popelka assisted by Eliza Stout assisted with the statistical data analysis and contributed to writing the manuscript. Anna Haw assisted with experimental work, data collection and edited the manuscript. Michele Miller, my co-supervisor, assisted with data collection and interpretation,

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As supervisors of the candidate, we confirm that Peter Buss has described the roles of his co-authors accurately, and that he functioned as the principal investigator for all three studies.

Leith Meyer: _____ Date: 10/7/17

Andrea Fuller: _____ Date: _____

LIST OF ABBREVIATIONS

A-a	-	alveolar-arterial
α	-	alpha
ATP	-	adenosine triphosphate
β	-	beta
BE_{ecf}	-	base excess
BM	-	body mass
BötC	-	Bötzinger complex
BTPS	-	body temperature and saturated pressure
°C	-	degree Celsius
Ca	-	calcium
cAMP	-	cyclic adenosine monophosphate
CI	-	confidence interval
CNS	-	central nervous system
CO_2	-	carbon dioxide
CSF	-	cerebrospinal fluid
D	-	dopamine
DOR	-	delta-opioid receptor
Δ	-	delta
DMSO	-	dimethyl sulphoxide
DRG	-	dorsal respiratory group
E	-	east
ET	-	endotracheal
F_EO_2	-	expired oxygen fraction
Fig.	-	Figure
F_IO_2	-	inspired oxygen fraction
5-HT	-	5-hydroxytryptamine
$F_{ET}O_2$	-	end-tidal oxygen fraction
f_R	-	respiratory rate
H	-	histamine
HBR	-	Hering-Breuer reflex

HCO_3^-	-	bicarbonate
IM	-	intramuscular
i.m.	-	intramuscular
IV	-	intravenous
i.v.	-	intravenous
IU	-	international units
IQR	-	interquartile range
κ	-	kappa
kg	-	kilogram
KOR	-	kappa-opioid receptor
K	-	potassium
L	-	litre
mg	-	milligram
min	-	minute
ml	-	milliliter
mm	-	millimeter
mm Hg	-	millimeters of mercury
mM	-	millimole
mmol	-	millimole
MOR	-	mu-opioid receptor
M	-	muscarinic
n	-	number
Na	-	sodium
NMDA	-	N-methyl-D-aspartate
NTS	-	nucleus tractus solitarius
$P(A\text{-}a)\ O_2$	-	alveolar-arterial oxygen partial pressure gradient
$PaCO_2$	-	arterial partial pressure of carbon dioxide
PaO_2	-	arterial partial pressure of oxygen
Pb	-	barometric pressure
P_B	-	barometric pressure
$P\bar{E}CO_2$	-	mixed-expired carbon dioxide pressure
$P_{ET}CO_2$	-	end-tidal carbon dioxide pressure

P_{H2O}	-	water vapour pressure
P_{H2O}	-	water vapour pressure
pre-BötC	-	pre-Bötzinger complex
Q	-	quartile
RTN	-	retrotrapezoid nucleus
RTN/pFRG	-	retrotrapezoid nucleus/parafacial respiratory group
rVRG	-	rostral ventral respiratory group
S	-	south
SaO_2	-	arterial haemoglobin oxygen saturation
SD	-	standard deviation
STPD	-	standard temperature and dry pressure
t	-	time
Tb	-	body temperature
T_B	-	body temperature
μ	-	mu
$\dot{V}_{CO_2}$	-	carbon dioxide production
$\dot{V}_{DPHYS}$	-	physiological deadspace
$\dot{V}_{EXP}$	-	expected respiratory minute volume
$\dot{V}_{O_2}$	-	oxygen consumption
$\dot{V}_E$	-	expired minute ventilation
VRC	-	ventral respiratory column
V_T	-	tidal volume
$\dot{V}/\dot{Q}$	-	ventilation/perfusion ratio
%	-	percent

Note: some abbreviations varied due to format for the journal

CHAPTER 1

Introduction

South Africa, as in many other countries around the world, has in recent history suffered significant losses in numbers, distribution, and diversity of its wild mammals. The arrival of European colonists and increased agricultural requirements drastically changed animals' habitats. Loss of suitable habitat, frequently compounded by excessive hunting and the introduction of non-indigenous diseases such as the Rinderpest panzootic of the late 19th century, resulted in the local extinction of some species (Joubert 2007; Thomson 2006). Hunting pressure is believed to have resulted in the extinction of the historical population of blue antelope (*Hippotragus leucophaeus*) (Kerley *et al.* 2009).

In the late-1800s, it was realized that without some form of protection, the natural heritage of the country would suffer irreparable damage, and this resulted in the proclamation of the area between the Sabie and Crocodile Rivers as a game reserve in 1889. This was the first step in the process which led to the establishment of the Kruger National Park in 1926 (Joubert 2007). Since this initial beginning, numerous national parks and provincial game reserves have been established throughout the country. There has also been a dramatic increase in the number of private game farms, many of which are used for commercial purposes including photographic safaris, hunting and breeding of endangered species (Van der Merwe & Saayman 2003). Although there has been an increase in land areas supporting wildlife, many of these are isolated either by distance, fences or, in some cases, regulatory disease control measures.

Requirements for species reintroductions into former ranges, management of non-contiguous populations, capture and translocation of animals for commercial purposes, and captive breeding of "high-value" species has resulted in the development of wildlife chemical capture techniques (La Grange, Du Toit & Van Rooyen 2016). In mega-herbivores, including the southern white rhinoceros (*Ceratotherium simum simum*), these techniques are based on the use of etorphine, a potent opioid, combined with either a tranquillizer or sedative. Although these drug combinations are commonly used in white rhinoceros immobilization, significant respiratory depression and cardiovascular perturbations occur

(Boardman *et al.* 2014; Haw *et al.* 2014, 2015; Miller *et al.* 2013; Wenger *et al.* 2007). As the white rhinoceros is listed as near threatened, with a dramatic escalation in poaching in recent years, it is essential that methods for mitigating these negative physiological consequences are investigated to improve the safety of capture procedures (Boardman *et al.* 2014; Emslie 2011). This chapter reviews the requirements for chemical restraint in white rhinoceros, historical development of currently used immobilizing drug combinations, and introduces the cardiorespiratory side-effects in immobilized rhinoceros and interventions used to manage them.

1.1 History of white rhinoceros chemical immobilization

Chemical capture of white rhinoceros is an essential procedure used for the conservation and management of this species (Haw *et al.* 2014). The initial impetus for developing capture and transport techniques, including immobilizing drug cocktails, was the requirement in the early 1960s to reintroduce this mega-herbivore back into its former historical range from a single remaining population in South Africa (Condy 1964).

By 2004, the translocation of white rhinoceros had resulted in 293 additional separate populations with an estimated total population in excess of 10,000 animals being established in South African state and municipal reserves, in other African countries, on private land and in zoological gardens across the globe (Friedman & Daly 2004; Rookmaaker 2000). According to the World Wildlife Fund (n.d.), the population had increased to over 20,000 individuals by 2014. More recently, chemical immobilization has been used to combat a marked increase in poaching with rhinoceros captured for the removal of horns, implantation of identifying microchips or translocation to safer areas (Boardman *et al.* 2014; Bush *et al.* 2004; Kock *et al.* 1995). Chemical restraint is also essential for veterinary examination of individuals, performing medical procedures, reproductive interventions and collection of biological samples (Haw *et al.* 2015; Heard, Olsen & Stover 1992; Miller *et al.* 2013; Waltzer *et al.* 2000; Wenger *et al.* 2007). All southern white rhinoceros alive today are ultimately

derived from stock originating in Hluhluwe-iMfolozi Park, KwaZulu-Natal. By the end of the 19th century, it is estimated this single population had been reduced to about 200 animals (Rookmaaker 2000). By the early 1960s, there were approximately 650 rhinoceros which occurred in this park or roamed on adjacent Crown land. These animals were vulnerable to ever-increasing land invasions by squatters and a lack of food due to potential drought. To safeguard these rhinoceros, it was decided that approximately 400 animals should be removed and translocated elsewhere (Harthoorn 1962).

The drug combinations used in immobilizing the first rhinoceros were based on diethyl-thiambutene (Themalon), a synthetic opioid, in various combinations with morphine, l-hyoscine (Scopolamine), phencyclidine (Sernyl), and chlorpromazine (Largactil) (Anton-Stephens 1954; Brown & Laikin 2011; Cohen 1962; Haigh 1990; Harthoorn 1962). Mean induction times were 15 to 20 minutes (min), and distances travelled between drug administration and immobilization ranged between 274 meters and 5.6 kilometers in the twelve animals initially darted. These times and distances would be considered unacceptable by today's standards; however, all animals survived (Harthoorn 1962). Other disadvantages were that the Themalon had to be dissolved just prior to use and large volumes necessitated the use of large darts for an adult rhinoceros (Haigh 1990). Despite these early challenges, eight juvenile rhinoceros were subsequently captured in 1962 with the same dose of Themalon, Scopolamine and Largactil and successfully translocated to Zimbabwe (Condy 1964).

The advent of etorphine (M99), a semi-synthetic molecule derived from thebaine, with a marked increase in potency compared to Themalon, allowed for use of much smaller volumes in the immobilization of large herbivores. The increase in potency, compared to morphine, is due to its molecular structure which has a greater affinity for opioid receptors and increased lipid solubility, allowing it to pass more effectively through the blood-brain-barrier (Haigh 1990). Etorphine is now the most commonly used opioid in the chemical immobilization of rhinoceros; although, it has been used in combination with various sedatives and

tranquillizers (Burroughs *et al*, 2012b; Portas 2004). Early reports indicate the successful use of etorphine alone in the immobilization of captive rhinoceros for medical examination and treatment (Heard *et al.* 1992; Le Blanc *et al* 1987). Carfentanil, the most potent of the opioids, has been used to immobilize rhinoceros in the past; however, it is not commercially available in southern Africa (Portas 2004; Raath 1999).

By 1993, the drug cocktails used by the then Natal Parks Board had evolved from Themalon-based combinations to using etorphine and fentanyl in a ratio of 1:10 (mg) with hyoscine added. Fentanyl, a synthetic opioid, was included as it resulted in quicker and smoother inductions, and reduced muscle tremoring and rigidity in the immobilized animal. Hyoscine, a parasympatholytic, was purported to induce mydriasis with temporary blindness causing the animal to stop running sooner (Haigh 1990; Rogers 1993). Rogers (1993) also suggested that azaperone, a butyrophenone tranquillizer, could be incorporated into the immobilizing drug mixture, and some operators included hyaluronidase or dimethyl sulfoxide (DMSO) to improve drug absorption and reduce induction times. Hyaluronidase is an enzyme which breaks down hyaluronic acid between cells and DMSO is a carrier agent which promotes drug absorption (Burroughs, Meltzer & Morkel. 2012a; Plumb 2008). Around this time in the Kruger National Park, etorphine plus azaperone was becoming the combination of choice. Hyoscine was not used due to the possible deleterious side-effects of parasympatholytic drugs, including tachycardia and extended gastrointestinal stasis (Brown & Laikin 2011; Hattingh, Knox & Raath 1994; Raath 1999).

In the mid-1990s, 141 free-ranging rhinoceros were immobilized in Zimbabwe as part of a conservation study using etorphine alone or combinations of etorphine with xylazine, fentanyl or detomidine. Hyaluronidase was added to all combinations to reduce induction times (Kock *et al.* 1995). Xylazine and detomidine are alpha $(\alpha)_2$ - receptor agonists resulting in sedation (Burroughs *et al.* 2012a). A significant difference between the immobilization of these animals and those in the early days of capture was a reduced average induction time of

about 6 min compared to 15 to 20 min (Harthoorn 1962; Kock *et al.* 1995). Induction times greater than 6 min were considered to be a medical emergency due to the risks of hyperthermia and metabolic acidosis, associated with prolonged running. Etorphine plus detomidine was found to be the preferred combination as it resulted in a rapid and smooth induction, superior muscle relaxation, and absence of muscle rigidity and limb paddling. Heart rates in rhinoceros immobilized with etorphine and detomidine were also significantly lower compared to those in rhinoceros immobilized with the other drug combinations. However, it was noted that all combinations produced some degree of cardiovascular and respiratory system compromise (Kock *et al.* 1995).

In 2000, Radcliffe, Shannon & Childs (2000) reported the use of butorphanol, a mixed synthetic agonist-antagonist opioid, combined with azaperone for the chemical restraint of captive rhinoceros. The combination resulted in varying levels of neuroleptanalgesia, with some rhinoceros remaining standing and others becoming recumbent. The combination also allowed for minor surgical procedures. However, total volumes of both drugs were beyond the capacity of available darts and this combination was not tried on free-ranging individuals. Butorphanol has also been used in combination with etorphine, acepromazine and detomidine for chemical restraint with supplementary doses of both ketamine and xylazine administered intravenously (IV) in captive rhinoceros to facilitate reproductive evaluation, semen collection, and artificial insemination (Waltzer *et al.* 2000).

In the last ten years, etorphine plus azaperone has become the drug combination routinely used in the immobilization of boma-adapted and free-ranging white rhinoceros of both sexes and all ages (Boardman *et al.* 2014; Burroughs *et al.* 2012b; Bush *et al.* 2004; Haw *et al.* 2014; Miller *et al.* 2013). Etorphine is commonly used as it has potent immobilizing effects so low volumes can be administered by dart. Azaperone has a synergistic effect and is added to the combination to reduce induction time and improve immobilization quality (Boardman *et al.* 2014; Portas 2004; Wenger *et al.* 2007). Onset of effects is rapid

following drug administration and free-ranging rhinoceros darted from a helicopter become recumbent within approximately 4 to 7 min, and boma-housed rhinoceros within 5 to 6 min (Haw *et al.* 2015; Miller *et al.* 2013). Free-ranging rhinoceros are frequently given higher doses to ensure induction times are not prolonged (Miller *et al.* 2013). Midazolam, a benzodiazepine sedative which produces excellent muscle relaxation, has recently been investigated as an immobilizing drug cocktail adjunct (Van Zijll Langhout *et al.* 2016).

Potent opioids, such as etorphine, are the only class of drugs capable of inducing rapid and reversible immobilization in white rhinoceros (Haw *et al.* 2015). However, marked respiratory depression is a common side-effect and may lead to hypoxaemia, hypercapnia, acidaemia and complications associated with decreased tissue oxygenation (Boardman *et al.* 2014; Haw *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013; Wenger *et al.* 2007). Cardiovascular effects of this potent opioid are less studied but include tachycardia and hypertension (Hattingh *et al.* 1994; Heard *et al.* 1992; LeBlanc *et al.* 1987; Raath 1999; Waltzer *et al.* 2000). These potentially fatal complications have been managed in the past by the administration of partial opioid antagonists and tracheal oxygen insufflation (Burroughs *et al.* 2012b; Bush *et al.* 2004; Radcliffe & Morkel 2007; Radcliffe *et al.* 2000). Opioid partial antagonists that have been used include diprenorphine, nalbuphine and, more commonly, nalorphine (Bush *et al.* 2004; Portas 2004; Raath 1999; Wenger *et al.* 2007). Due to limited and variable responses, and a lack of availability of some of these agents, especially nalorphine, butorphanol has recently become widely used as a partial antagonist to improve respiratory function (Haw *et al.* 2014; Miller *et al.* 2013).

The following chapters will review the physiology of respiration and cardiovascular control mechanisms, how these are influenced by etorphine and azaperone, and the management of cardiopulmonary side-effects, due to immobilization with the use of butorphanol emphasized.

1.2 Respiratory system

Breathing provides the body with oxygen for aerobic metabolism and eliminates carbon dioxide produced during the metabolism of organic compounds (Taylor & Weibel 1981). It requires continuous movement of skeletal muscles and consumes approximately 7% of metabolic output in animals at rest (Feldman, Del Negro & Gray 2013). Rhythmic contractions of the diaphragm, intercostal and abdominal muscles cause changes in lung volume and movement of air in and out of the respiratory airways (Bianchi, Danavit-Saubié & Champagnat 1995). The diaphragm, a defining characteristic of mammals, is an unusually powerful muscle involved in changing thoracic volume which allows for the high levels of ventilation required with increases in metabolism (Feldman *et al.* 2013; Feldman & Negro 2006).

1.2.1 Respiratory system control

Inspiration in the non-exercising animal is an active process with contraction of the diaphragm and external intercostal muscles to inflate the lungs, and expiration is passive as these muscles relax and the ribs return to their original position due to elastic recoil. As activity increases and consequently the metabolic demands, so expiration also becomes active with contraction of abdominal and internal intercostal muscles (Feldman *et al.* 2013). The brainstem produces efferent signals which generate and control the movements of breathing via motor neurons which innervate the various muscles involved in breathing (Bianchi *et al.* 1995; Rekling & Feldman 1998). These outputs are modulated by central and peripheral chemoreceptors which sense pH changes and blood oxygen and carbon dioxide levels, conscious inputs from the brain cortex, and sensory neurons within the lungs, muscles and blood vessels (Mitchell & Johnson 2003; Pattinson 2008).

The respiratory neural control system not only has to be highly robust as breathing is a continuous homeostatic process but also sufficiently flexible to accommodate rapid fluctuations in metabolic demand and other short-term perturbations including postural changes, suckling, swallowing, sniffing, chewing and

vocalization (Bianchi *et al.* 1995; Mitchell & Johnson 2003; Smith *et al.* 2007). The system also needs to be sufficiently flexible to accommodate changed circumstances such as persistent hypoxia, injury, disease and aging (Mitchell & Johnson 2003).

The rhythm of inspiration and expiration is generated by a pontine-medullary respiratory network which coordinates the activity of spinal and cranial motor neurons (Smith *et al.* 2007). These neurons include the phrenic nerve which innervates the diaphragm, branches of the vagus nerve which supply the pharyngeal muscles and bronchial smooth muscle, recurrent laryngeal nerves of the larynx, and nerves arising from the spinal column which innervate the intercostal and abdominal muscles (Bianchi *et al.* 1995; König, Liebich & Červeny 2009). Pharyngeal muscles are important in maintaining upper airway patency and laryngeal muscles regulate the diameter of the airway and resistance to airflow during inspiration and expiration (Haji, Takeda & Okazaki 2000). The glossopharyngeal nerve, hypoglossal nerve and branches of the facial and trigeminal nerves also take part in regulating muscle activity of the upper-respiratory tract during breathing (Bianchi & Gestreau 2009; Bianchi *et al.* 1995; König *et al.* 2009).

There are three essential phases of respiration: inspiration, in which respiratory muscles contract; post-inspiration, in which inspiratory muscles progressively stop contracting and adductor muscles of the upper airway reduce inhalation; and late expiration in which expiratory muscles contract. In breathing at rest, expiration can be passive and there will not be motor outputs (Bianchi & Gestreau 2009; Haji *et al.* 2000; Koo & Eikermann 2011; Smith *et al.* 2007). The three phases correspond to phasic activity of respiratory neurons within the ventral respiratory column (VRC) located in the medulla. The VRC is modulated by the pons (Bianchi & Gestreau 2009; Haji *et al.* 2000; Koo & Eikermann 2011; Smith *et al.* 2007). It is believed that a rhythm generator is responsible for switching between the different respiratory phases, and a pattern generator shaping neuron activity (Haji *et al.* 2000).

Three areas arranged rostro-caudally are found in the VRC: Bötzinger complex (BötC), pre-Bötzinger complex (pre-BötC) and rostral ventral respiratory group (rVRG) (Smith *et al.* 2007). A neuronal kernel consisting of a network of excitatory neurons with pacemaker properties located in the pre-BötC is believed to generate an inspiratory rhythm and is essential in normal breathing (Feldman *et al.* 2013; Smith *et al.* 2000). The hybrid pacemaker-network model of respiratory rhythm generation suggests that these pacemaker cells receive tonic excitatory inputs and are interconnected, creating synchronized discharges and establishing a basic oscillation. Inhibitory synaptic connections with the kernel regulate the shape, duration and interval between discharges to create the dynamic range of inspiratory and expiratory network activity required in respiration (Rekling & Feldman 1998; Smith *et al.* 2000). It is uncertain if only the pre-BötC pacemaker is responsible for generating respiratory rhythm or it is part of a collective of pacemaker neurons (Koo & Eikermann 2011). Motor output patterns of the phrenic nerve originate from and are driven by the pre-BötC (Smith *et al.* 2007). The BötC is a major source of expiratory activity in the respiratory network and provides phasic inhibition of pre-BötC inspiratory activity. Pontine activation of the BötC during inspiration provides strong modulation of the VRC and seems critical in rhythm and pattern generation (Smith *et al.* 2007)

With increased physical activity, there is a transition from passive to active expiration to increase ventilation in response to metabolic demands and it has been proposed that a group of neurons is responsible for driving expiration. Stimulation of a region, termed the retro-trapezoid nucleus/parafacial respiratory group (RTN/pFRG), nearby the pre-BötC, results in active expiration (Feldman *et al.* 2013). It is proposed the RTN/pFRG (expiration) interacts with the pre-BötC neurons (inspiration) as a coupled oscillator system in the regulation of respiratory rhythm (Feldman & Del Negro 2006; Koo & Eikermann 2011). Although the mechanism of activation is unknown, the RTN is involved in central chemoreception that regulates respiration depending on carbon dioxide levels in the blood (Smith *et al.* 2007). In expiration, regardless of the signal origin, there is a rapid decline in phrenic nerve activity, inspiratory airflow stops and expiratory

airflow begins (Feldman *et al.* 2013). The pontine respiratory group, which includes the parabrachialis medialis and Kolliker-Fuse nucleus, is believed to be associated with respiratory volume control (Dahan, Aarts & Smith 2010).

Hypercapnia is the primary driver of respiration and the ventilatory response to hypoxia is considered a vital backup reflex. Respiratory rhythmogenesis and regulation of tonic drive to respiratory motor outputs is moderated by hypercapnic stimulation of central chemosensitive structures. A 1mm Hg partial pressure increase in carbon dioxide increases ventilation by 20% to 30% (Koo & Eikermann 2011). Multiple chemosensing areas located mostly within the brain stem make up the central chemoreceptor, and include the nucleus tractus solitarus (NTS), midline medullary raphe, pre-BötC and RTN/pFRG (medulla), locus coerulus (pons), and fastigial nucleus (cerebellum) (Haji et al. 2000; Pattinson 2008). The contribution of these different areas in chemosensing may vary depending on the age and state (e.g., sleep-awake, rest-exercise) of an animal (Feldman *et al.* 2013). The mechanisms underlying central chemosensitivity are not fully understood. It is hypothesized that a distinct population of astrocytes sense changes in pH and control the activity of nearby chemosensitive neurons, including the RTN/pFRG, through the release of adenosine triphosphate (ATP) which in turn modulates respiration (Feldman *et al.* 2013). A decrease or increase in pH of the brain interstitial fluid, which bathes the central chemoreceptor increases or decreases ventilation, respectively. As carbon dioxide moves freely through the blood-brain barrier into the cerebrospinal fluid (CSF), increases in arterial carbon dioxide tension result in a decrease in pH following the formation of carbonic acid which dissociates into hydrogen and bicarbonate ions. As the CSF communicates directly with the interstitial fluid, this increase in acidity stimulates breathing (Robinson 2007).

Peripheral chemoreceptors, especially the carotid bodies, sense changes in arterial oxygen levels and relay this information to those areas of the brain regulating respiration (Prabhakar 2000). Carotid body Type I (glomus) cells are the primary sensors of hypoxia, resulting in impulses that travel via the carotid sinus branch of

the glossopharyngeal nerve, which terminates almost exclusively in the NTS (Haji *et al.* 2000; Koo & Eikermann 2011; Pattinson 2008,). A direct link between the NTS and RTN facilitates modulation of respiration.

In humans, the carotid bodies are essential in mediating the hypoxic ventilatory response (Pattinson 2008). Hypoxia is thought to cause the release of an excitatory neurotransmitter from glomus cells, which results in an increased sensory discharge from afferent nerve endings with a haem and/or redox-sensitive enzyme and/or a K^+ channel protein being the primary oxygen sensor. It is likely that additional sensory mechanisms become involved as the severity of hypoxia increases. It is proposed that inhibitory transmitters also released by glomus cells prevent over-excitation and assist in maintaining a sustained response during prolonged periods of hypoxia (Prabhakar 2000). Glomus cells also sense carbon dioxide and the response to hypoxia is influenced by arterial levels of carbon dioxide; a synergistic response between hypercapnia and hypoxia occurs (Pattinson 2008; Prabhakar 2000). In dogs, increased carbon dioxide in the carotid arteries excites expiratory neurons influencing the expiratory phase of respiration (Haji *et al.* 2000).

The Hering-Breuer reflex (HBR) prevents over-inflation of the lungs and primarily controls transition from inspiration to expiration. This reflex relies on slowly adapting stretch receptors in pulmonary tissue which are activated by lung inflation and transmit information to brainstem neurons controlling the duration of inspiration and expiration (Haji *et al.* 2000; Koo & Eikermann 2011). An active HBR limits inspiratory duration and tidal volume, and increases respiratory frequency (Koo & Eikermann 2011).

1.2.2 Respiratory effects of immobilizing drugs

1.2.2.1 Opioids

Opioids include both opiates, which are derivatives of opium, and drugs that are synthetic or semi-synthetic agents but also act on opioid receptors. Opioids have been used extensively as analgesics in human medicine dating back several millennia, and since the early 20th century, by veterinarians, in treating a variety of conditions including colic in horses, coughs, and for analgesia (Feng et al. 2012; Kukanich & Papich 2009).

Opioids act on an endogenous opioidergic system and their effects are mediated through three major opioid receptor families, mu-opioid receptor (MOR); delta-opioid receptor (DOR); and kappa-opioid receptor (KOR). A fourth receptor type is also recognized, nociception or orphanin FQ receptor or the opioid receptor-like orphan receptor. A sigma receptor has been suggested in the past but has since been disregarded due to a lack of sensitivity to the opioid antagonist naloxone (Feng *et al.* 2012; McDonald & Lambert 2005). Synthetic opioids have a relatively high selectivity to MOR, DOR and KOR, which are similar in their primary structures, function and intracellular signaling mechanisms (Feng *et al.* 2012).

There is significant pharmacological evidence which supports the concept of multiple opioid receptor subtypes (MOR_{1-3}, DOR_{1-2}, and KOR_{1-3}) and that these subtypes are responsible for different clinical effects, e.g. MOR_1-subtype produces analgesia and MOR_2-subtype produces respiratory depression. However, the molecular evidence for identification of subtype-specific genes to support this sub-classification of receptors is lacking (Dietis, Rowbotham & Lamber 2011; Feng *et al.* 2012).

Opioid receptors are classified as seven transmembrane spanning G-protein coupled receptors. Binding of opioid receptors results in the inhibition of adenylyl

cyclase and a reduction in intracellular levels of cyclic adenosine mono-phosphate (cAMP). This reduction causes changes in neuronal cell polarization, reduction in impulse transmission and inhibition of neurotransmitter release (Boom *et al.* 2012; McDonald & Lambert 2005). Other intracellular signal pathways may be triggered resulting in activation or inhibition of various cellular proteins and signaling mechanisms, which could explain the multiple biological outcomes associated with opioid administration (Boom *et al.* 2012). It is possible that opioids have effects on receptors other than opioid receptors. Fentanyl has been shown to inhibit muscarinic (M)-receptor activation by acetylcholine, resulting in vasodilation (Koo & Eikermann 2011).

Opioid receptors not only exist in the nervous system but also in peripheral organs, and the distribution of receptors and receptor types may vary between organs and different animal species (Feng *et al.* 2012). Opioid receptors are located in multiple components of the respiratory system. Both MORs and DORs are found in the respiratory control centres of the medulla and pons, and higher respiratory centres in the insula, thalamus and anterior cingulate cortex. Opioid receptors are also found in the vagal nerves and carotid bodies of the peripheral chemoreceptors, and mechanosensory receptors in the epithelium, submucosa and muscular layers of the airways (Koo & Eikermann 2011; Pattinson 2008).

MORs are located in the pre-BötC and administration of opioids suppresses pre-BötC activity (Boom *et al.* 2012; Pattinson 2008,). Those neurons are preferentially inhibited during inspiration (Koo & Eikermann 2011). Opioid activity at the parabrachialis medialis and Kolliker-Fuse nuclei in the pons is also thought to influence output from the pre-BötC and contribute to irregular respiratory rhythm. Activity of the RTN/pFRG is not influenced by the presence of opioids and studies suggest this group of neurons takes over respiratory rhythm generation when the pre-BötC becomes depressed, and contributes to the alteration in respiratory rhythm (Pattinson 2008). Opioids result in profound depression of the respiratory response to increased arterial carbon dioxide level (hypercapnic ventilatory response); a response primarily modulated by the central

chemoreceptors. Peripheral chemoreceptor carotid body activity is inhibited by opioids and increased ventilation in response to hypoxia (hypoxic ventilatory response) is suppressed. The hypercapnic ventilatory response may also be influenced as glomus cells sense carbon dioxide (McDonald & Lambert 2005; Pattinson 2008). It has also been suggested that opioids may inhibit the NTS, which receives afferent inputs from the carotid bodies (Pattinson 2008).

As well as blunting the response of central and peripheral receptors to hypercapnia and hypoxia, opioids also decrease the central drive to the muscles which drive respiration and dilator muscles of the upper airways (Koo & Eikermann 2011). Opioid administration is associated with an increase in chest wall and abdominal muscle rigidity, and a decrease in phrenic nerve and diaphragmatic muscle activity, which contribute to a decrease in ventilation (Koo & Eikermann 2011). Inhibition of the hypoglossal motor neuron and reduced innervation of the genioglossus muscle can result in potentially fatal upper airway obstruction. Opioids influence the function of vagal laryngeal abductor and pharyngeal constrictor motor neurons, further contributing to upper airway resistance. Activity of the tongue can be suppressed centrally (Koo & Eikermann 2011). Bronchoconstriction due to opioids may limit airflow within the respiratory tree and influence alveolar ventilation (Koo & Eikermann 2011).

Studies in "knockout" mice lacking MOR have confirmed that this group of receptors is largely responsible for mediating opioid-induced respiratory depression (Dahan *et al.* 2010). Opioids have less of a depressant respiratory effect at DOR and no effect when KOR is activated (McDonald & Lambert 2005; Feng *et al.* 2012). It also appears that interactions between the opioid receptors influence the extent of respiratory depression. For example, opioid activities at DOR and MOR are synergistic in suppressing respiration with no interaction occurring with the activation of both KOR and MOR (Feng *et al.* 2012). Pharmacological receptor binding studies have ascribed respiratory depression to the MOR_2-subtype, and opioid-induced analgesia to the MOR_1-subtype; however, as indicated earlier, molecular cloning studies have only identified a single

encoding gene for each of the main opioid receptor groups (Boom *et al.* 2012; Haji *et al.* 2000; McNally & Akil 2002).

Clinically, respiratory depression due to opioids is characterized by a bradypnoea and an irregular rhythm with a decrease in minute and tidal volumes resulting in hypercapnia and hypoxaemia (Boom *et al.* 2012; West 2008). Changes in respiratory rhythm and pattern tend to occur at lower opioid doses and a reduction in tidal volume as the dose increases (Pattinson 2008). At high opioid doses, apnoea and death may result (Feng *et al.* 2012).

Etorphine

Etorphine is an opioid agonist at MOR, DOR and KOR and is used extensively in the chemical capture of both captive and free-ranging herbivores (Branson & Gross 2001; Burroughs *et al.* 2012b; Haigh 1990; Yaksh & Wallace 2011). Etorphine is the only opioid currently used in the chemical capture of free-ranging white rhinoceros (Branson & Gross 2001). The principle side-effect of etorphine in immobilized wildlife is respiratory depression with both rate and minute volume affected (Haigh 1982). However, the degree of depression is dependent on species, dose rate and other drugs administered in combination (Burroughs *et al.* 2012b).

The high potency of etorphine compared to other opioids, allows for a sufficient dose to be delivered by dart in the immobilization of a wide variety of animals including the mega-herbivores. However, there are considerable differences in sensitivity between species with elephants, hippopotamus and rhinoceros considered more sensitive and equids less sensitive to etorphine. Doses per unit body mass of etorphine that result in immobilization in antelope vary widely between species (Burroughs *et al.* 2012b; Haigh 1990). Etorphine is seldom administered on its own, and usually combined with synergistic drugs to reduce the time from administration to recumbency and improve the quality of immobilization. Alpha$_2$-adrenergic agonists (xylazine, detomidine and medetomidine) and butyrophenones (azaperone) have been used in combination

with etorphine in the chemical capture of white rhinoceros (Haigh 1990; Kock *et al.* 1995; Miller *et al.* 2013; Wenger *et al.* 2007).

Butorphanol

Butorphanol is a synthetic mixed opioid agonist-antagonist analgesic agent which is pharmacologically related to the agonist-antagonist drugs pentazocine and buprenorphine (Gillis, Benfield & Goa 1995; Commiskey, Fan & Rockhold 2005; WHO Expert Committee on Drug Dependence 2006). The drug was originally used as an antitussive agent in dogs and more recently, it has been approved as an analgesic in cats and horses (Grimm & Lamont 2007).

Butorphanol binds to the three principle opioid receptors, MOR, DOR and KOR with an affinity ratio of 1:4:25, and produces complex effects due to either MOR antagonist or low-efficacy MOR agonist and KOR agonist activities (Commiskey *et al.* 2005; Haw *et al.* 2015; Vivian *et al.* 1999). The seemingly variable effects of butorphanol may in part be due to differences in affinities for and efficacies at the different opioid receptors (Vivian *et al.* 1999). Opioid receptor binding studies suggest that butorphanol has a moderate affinity and no activity at MOR, and high affinity and moderate activity at KOR (McCrackin *et al.* 1994).

Early studies suggested that butorphanol, as a mixed opioid agonist-antagonist, did not produce significant respiratory depressant effects due to a 'plateau or ceiling effect'; however, there is now contradictory evidence which suggests marked respiratory perturbations can occur (WHO Expert Committee on Drug Dependence, 2006). Respiratory effects of butorphanol are variable depending on species, dosage and presence of other drugs. For example, butorphanol in monkeys resulted in respiratory depression (Vivian *et al.* 1999); a dose of 0.4 mg/kg IV did not alter cardiorespiratory function in the horse (Sellon *et al.* 2001); in detomidine–sedated horses, the addition of butorphanol decreased ventilation (Miller *et al.* 2013); in dogs, the central chemoreceptor threshold to carbon dioxide was raised (Plumb 2008); butorphanol (0.3 mg/kg IM) in rabbits reduced

respiratory rate but there were no significant changes in arterial blood gas tensions (Schroeder & Smith 2011).

Butorphanol has been used in the past 10 years to selectively antagonize the marked respiratory depression that occurs in etorphine-immobilized white rhinoceros (Haw *et al.* 2014; Wenger *et al.* 2007). MOR_2-subtype mediates respiratory depression and stimulation of KOR induces sedation as well as spinal analgesia (McCrackin *et al.* 1994). Etorphine as a pure opioid agonist acts on MOR (MOR_1 and MOR_2) and KOR resulting in respiratory depression and immobilization of the animal (Yaksh & Wallace 2011). Therefore, it was hypothesized that butorphanol would selectively antagonize the MOR_2-subtype and thereby limit the adverse respiratory effects of etorphine; however, sedation in the immobilized rhinoceros would not be reversed due to the agonist activity at KOR (McCrackin *et al.* 1994; Wenger *et al.* 2007).

1.2.2.2 Butyrophenones

Azaperone and haloperidol are butyrophenones used as tranquillizers in wildlife. Azaperone is a short-acting drug, lasting 2 to 3 hours, and is frequently administered in combination with etorphine in the immobilization of rhinoceros. Haloperidol's duration of clinical effect is up to 18 hours; however, due to the formation of precipitates, it cannot be mixed with etorphine (Burroughs *et al.* 2012b; Swan 1993). Azaperone modifies behaviour in animals primarily by central dopamine (D) receptor blockade. Azaperone has been shown to bind with D_2-receptors; however, it is uncertain if it binds with other dopamine receptors (Leysen & Gommeren 2008). This tranquillizer also has low binding affinities for other receptor types including α_1-, histamine $(H)_1$-, 5-hydroxytryptamine- (5-HT-) and M_3- receptors (Burroughs *et al.* 2012a; Lemke 2007).

Dopamine receptors are classified as D_1-like receptors which include the D_1- and D_5- subtypes, and D_2-like receptors which include the D_2-, D_3- and D_4- subtypes (Sugita *et al.* 2015). Dopamine binding at D_1-like receptors increases intracellular cAMP synthesis via stimulation of D-sensitive adenylate cyclase whereas D_2-like

receptors do not stimulate adenylate cyclase activity (Kolesnikova & Serebrovskaya 1998). D_1 receptors are found in multiple structures of the brain including the nucleus tractus solitarus (NTS) and locus coerulus of the central chemoreceptor and parabrachial nuclei of the pons (Haji *et al.* 2000; Iwase *et al.* 2013; Pattinson 2008). The parabrachial complexes are thought to participate in respiratory volume control and respiratory phase transition (Dahan *et al.* 2010; Iwase *et al.* 2013; Koo & Eikermann 2011). D_2-receptors are similarly found in various areas of the brain; however, they appear to have limited effects on the central respiratory neuronal network (Tsuchiya *et al.* 2011). Both D_1- and D_2-receptors are present in carotid bodies (Iwase *et al.* 2013, Tsuchiya *et al.* 2011).

Endogenous dopamine is believed to have significant respiratory modulation effects in both central respiratory neurons and peripheral carotid bodies (Lalley 2008). Dopamine appears to be important in regulating respiration both at rest and during periods of exercise (Iwase *et al.* 2013). Several D-receptors are implicated, with binding of dopamine to D_1- and D_4-receptors slowing respiratory rhythm, and D_1-receptor activation centrally increasing the respiratory response to hypercapnia (Lalley 2008). D_2-receptors may play a role in peripheral chemoreceptor function during periods of hypoxia (Tsuchiya *et al.* 2011).

It is reported that dopaminergic mechanisms are important in modulating respiration both centrally and peripherally (Hsiao, Lahiri & Mokashi 1989); however the role of dopamine in respiratory regulation is not conclusive. Research indicates differences in outcomes between *in vivo* and *in vitro* studies, depending on dopamine dosage and route of administration, specific D-agonists or antagonists used, and species (Kolesnikova & Serebrovskaya 1998). For example, Hedner *et al.* (1982) concluded that in the CNS, dopamine has a tonic function in stimulating respiration, based on intracerebroventricular or systemic administration of D-agonists and antagonists in halothane-anaesthetized rats. However, Eldridge & Millhorn (1981) reported that CNS D-agonist activity may decrease respiratory frequency in rats. In cats, it has been reported that central antagonism of D-receptors decreases the respiratory response to hypoxia

(Smatresk, Pokorski & Lahiri 1983). In part, the differences between studies may be explained by variations in D-receptor subtype within brain areas and between central and peripheral chemoreceptors; and the ability of certain D-agonists and antagonists to pass through the blood-brain-barrier (Zapata & Zuazo 1982).

Exogenously administered dopamine, which does not readily cross the blood-brain-barrier, appears to have an inhibitory function in peripheral chemoreceptors in the cat, dog, and goat, depressing ventilation in these species (Haji *et al.* 2000; Smatresk *et al.* 1983). This catecholamine has also been shown to negatively influence respiration in humans (Smatresk *et al.* 1983). The activity of dopamine at D_2-receptors is believed to inhibit the hypoxic and hypercapnic responses of peripheral chemoreceptors in man and animals (Bascom *et al.* 1991). In contrast, *in vitro* studies suggest that dopamine is an excitatory neurotransmitter in the carotid bodies and natural stimuli such as hypoxaemia, hypercapnia or acidaemia result in dopamine release and increased activity of the carotid sinus nerve (Kolesnikova & Serebrovskaya 1998). It has been suggested that this excitatory effect is also mediated by D_2-receptors, which illustrates the apparent complexity of dopamine functions in the peripheral chemoreceptors. It is possible that the observed differences in responses to endogenous and exogenous dopamine are due to D_2- receptors with variable affinities for dopamine which occur extra-, pre-, or post-synaptically in carotid body glomus tissue. Depending on location, these receptors may also have different functions including autoregulation of dopamine release, explaining why relatively large doses of exogenous dopamine have an inhibitory effect (Kolesnikova & Serebrovskaya 1998).

Reports on respiratory effects due to butyrophenones are largely limited to haloperidol as it has been used to investigate the role of dopamine in modulating respiration (Hedner *et al.* 1982; Hsiao *et al.* 1989; Smatresk *et al.* 1983; Zapata & Zuazo 1982). Haloperidol is a lipophilic D-antagonist which can penetrate the blood-brain barrier and is frequently used for medium duration (8 – 12 hours) tranquillization in wildlife (Burroughs *et al.* 2012a; Hsiao *et al.* 1989).

Butyrophenones appear to result in transient improvements in respiratory function with increases in respiratory minute volume, tidal volume and, or, respiratory rate. However, these improvements are variable between species with no response to haloperidol observed in humans, an increase in respiratory rate in dogs and cats, and improved ventilation in awake hypoxic goats and anaesthetized rabbits (Smatresk *et al.* 1983; Zapata & Zuazo 1982). Reflex respiratory adjustments to changes in blood gases, for example, fluctuations in arterial carbon dioxide partial pressure, are believed to limit the duration of haloperidol-induced respiratory improvements (Zapata & Zuazo 1982). Butyrophenones appear to augment peripheral chemosensory drive by blocking inhibitory D-receptors which are normally tonically activated by the continuous release of endogenous dopamine. This effect may be reinforced by the unmasking of excitatory D-receptors in the glomus tissues which are no longer inhibited by endogenous dopamine (Zapata & Zuazo 1982). Results in anaesthetized cats suggest that although haloperidol stimulates carotid chemoreceptor activity, it reduces the ventilatory response to hypoxia but not to hypercapnia (Hsiao *et al.* 1989; Smatresk *et al.* 1983). It was concluded that haloperidol prevents the central integration of peripheral chemoreceptor activity, despite being increased, resulting in an attenuated hypoxic respiratory response. The central respiratory response to increased carbon dioxide is unchanged following haloperidol administration (Smatresk *et al.* 1983).

Activation of respiratory system H_1-receptors has been shown to increase pulmonary resistance and influence ventilation distribution (Ahmed *et al.* 1980). In rats, the depletion of central 5-HT results in a substantial and sustained hyperventilation and 5-HT precursors depressed respiratory rate, resting respiratory minute volume and ventilation response to $PaCO_2$ in cats (Armtjo & Flórez 1974; Olson, Dempsey & McCrimmon 1979). M_3-receptors on airway smooth muscle mediate the contraction of airways in animals and humans (Fryer & Jacoby 1998).

Azaperone

Azaperone is an antagonist at D-receptors and has some activity blocking peripheral α_1-receptors (Burroughs *et al.* 2012a). Azaperone is partially metabolized to azaperol, which has 4 – 30 times reduced pharmacological activity (Mestorino *et al.* 2013). Azaperone is frequently combined with etorphine as an opioid synergist in the immobilization of wildlife and in recent years, this drug combination has been used extensively in white rhinoceros (Portas 2004). Azaperone is reported as having little effect on pulmonary function (Lemke 2007). In domestic equids, azaperone results in insignificant changes to arterial blood pH, and oxygen and carbon dioxide tensions; however, changes in respiratory rate are equivocal with both an increase or a decrease reported (Lees & Serrano 1976; Serrano & Lees 1976). Improvements in ventilation may occur in pigs, horses, and humans due to azaperone and it has been suggested that azaperone may inhibit respiratory depression due to opioids and general anaesthetics (Radcliffe *et al.* 2000).

1.2.3 Respiratory effects of immobilizing drugs in white rhinoceros

Early reports in the literature indicate that opioid-immobilized white rhinoceros experienced adverse respiratory effects. Keep (1971) first proposed respiratory depression resulting from etorphine in rhinoceros; increased respiration was consistently observed in etorphine-immobilized rhinoceros following the administration of the opioid antagonist cyprenorphine. A report on an adult captive bull white rhinoceros immobilized with 2.8 mg etorphine indicated a resulting respiratory rate of 15 to 20 breaths per min (LeBlanc, 1987). This rate is similar to 16 to 23 breaths/min reported for standing, unrestrained captive white rhinoceros, however, the dose of etorphine used by LeBlanc (1987) was low compared to those currently used in adult male rhinoceros (Burroughs *et al.* 2012b; Citino & Bush 2007). Heard *et al.* (1992) reported a much lower respiratory rate, 2 to 6 breaths/min, in a captive adult female animal administered multiple etorphine doses, both IM and IV (total dose 2.75 mg), over 165 min. This immobilized individual was also hypercapnic ($PaCO_2$ = 69 mm Hg) and hypoxaemic (PaO_2 = 61 mm Hg). Hypoventilation, ventilation/perfusion

mismatching, shunting and lung atelectasis were proposed as possible contributing factors.

Arterial blood gas values similar to those in captive rhinoceros were recorded in early reports for free-ranging animals darted from a helicopter and immobilized with a combination of etorphine and azaperone ($PaCO_2$ = 68 mm Hg; PaO_2 = 57 mm Hg). Etorphine-induced respiratory depression was proposed as the cause of the abnormal blood gas values, since a similar effect had been recognized in other species (Hatting and Knox 1994). Kock *et al.* (1995) evaluated several drug combinations in the capture of 141 free-ranging white rhinoceros, including etorphine alone or in combination with fentanyl, xylazine or detomidine. The inclusion of azaperone with etorphine in the drug mixture was not assessed. Respiratory frequency was considered normal (8 to 12 breaths/min) for an immobilized rhinoceros; however, this was lower than for a non-immobilized animal (16 to 23 breaths/min) (Citino & Bush 2007). Respiration was also subjectively evaluated as shallow in animals immobilized with etorphine alone or the combinations. In some rhinoceros, especially large cows and bulls, there was a progressively worsening hypoxaemia, detected by cyanosis of arterial blood samples. All drug combinations resulted in mortalities (total = 5) including a single death in an animal immobilized with etorphine alone. It was concluded that hypoventilation and hypoxaemia contributed to the mortalities. Other contributing factors may have been pressure on the diaphragm associated with a large digestive tract, pulmonary shunting and progressive lung atelectasis. Etorphine-induced sympathetic stimulation resulting in peripheral vasoconstriction, tachycardia and muscle tremors may also have contributed to the apparent hypoxaemia (Kock *et al.* 1995).

In a review article, Portas (2004) suggested that respiratory depression in immobilized white rhinoceros was common and etorphine dose-dependent. A list of potential causes of hypoxaemia and hypercapnia included hypoventilation, pulmonary shunting, ventilation-perfusion mismatching, lung atelectasis and

thoracic muscular rigidity. Portas (2004) proposed that increased thoracic muscular rigidity reduced respiratory excursions.

Bush *et al.* (2004) reported that free-ranging white rhinoceros darted with etorphine and azaperone from a helicopter, then administered nalorphine and/or doxapram IV once immobilized, experienced severe hypoxaemia (35 ± 9 mm Hg) and moderate hypercapnia (62 ± 12 mm Hg). They postulated that the low arterial oxygen partial pressure was due to the higher opioid doses required to reduce induction times under field conditions, sensitivity of rhinoceros to etorphine, physiological alterations associated with recumbency in a large animal, and development of a ventilation/perfusion mismatching. The immobilized rhinoceros were also acidotic (pH = 7.171 ± 0.073) due to a combination of both metabolic and respiratory acidosis. The metabolic acidosis was probably due to lactic acid accumulation as a result of muscle activity prior to and after the helicopter chase associated with darting, and hypoxaemia during recumbency. The administration of nalorphine, an opioid agonist-antagonist used to lighten opioid immobilization and improve respiration, was shown to have a negligible effect. Impaired pulmonary gas exchange was suggested by Fahlman, Foggin & Nyman (2004) as the cause of hypoxaemia and hypercapnia in free-ranging rhinoceros immobilized with etorphine, azaperone and detomidine.

In 2007, the first report on the use of butorphanol to mitigate the adverse cardiopulmonary effects associated with immobilizing drugs in white rhinoceros was published (Wenger *et al.* 2007). Butorphanol was added to the immobilizing drug combination of etorphine, azaperone and detomidine in the dart. It was hypothesized that butorphanol, a MOR antagonist and KOR agonist, would attenuate respiratory depression due to MOR activation associated with etorphine. However in the study, butorphanol was found to have no beneficial effects when compared to control animals and both groups showed respiratory and metabolic acidosis, hypoxaemia and hypercapnia. The study indicated that hypoventilation combined with an elevated alveolar to arterial oxygen partial pressure gradient ($P(A\text{-}a)O_2$) was the most probable cause of a low PaO_2. PaO_2 values were higher

and $P(A\text{-}a)O_2$ gradients lower in calves compared to in older animals, and those in sternal compared to lateral recumbency. The differences between age groups may have reflected the variation in body size and, therefore, the size of the digestive tract, and the impact this may have had on pulmonary shunting (Wenger *et al.* 2007).

Miller *et al.* (2013) compared cardiorespiratory effects in free-ranging rhinoceros between butorphanol added to the dart and butorphanol administered IV once etorphine-azaperone immobilized rhinoceros became recumbent. These animals were darted from the air after a brief chase. Rhinoceros administered butorphanol in the dart took longer to come to a standstill after darting; however, they did not run further compared to the animals in the butorphanol IV group. It was proposed that the intensity of exercise was less in these animals resulting in decreased lactic acidosis and hyperthermia. Rhinoceros given butorphanol in the dart also tended to remain standing once immobilized and this position, compared to lateral recumbency, is believed to have facilitated ventilation. Intravenous butorphanol administration reduced tachycardia and hypercapnia in the recumbent rhinoceros. However, the decrease in hypercapnia was not associated with an improvement in hypoxaemia, suggesting improved ventilation but possibly a decrease in oxygen diffusion. Alternative explanations given for the increased $PaCO_2$ and lack of change in PaO_2 were ventilation / perfusion mismatching associated with recumbency and increased tissue oxygen demand following the exertion of capture. It was also hypothesized that butorphanol may cause muscle relaxation and reduced carbon dioxide production in immobilized rhinoceros. It was concluded that butorphanol improved metabolic rather than respiratory parameters in immobilized rhinoceros (Miller *et al.* 2013).

Boardman *et al.* (2014) reported contradictory results in that butorphanol IV in etorphine-azaperone immobilized semi-captive rhinoceros (not chased and darted from a stationary vehicle) resulted in improved $PaCO_2$, PaO_2 and SaO_2, and a reduction in pH. However, the rhinoceros were still more hypoxaemic (PaO_2 = 45.03 mm Hg) compared to rhinoceros prior to butorphanol administration in the

studies by both Wenger *et al.* (2007) (PaO_2 = 58.9) and Miller *et al.* (2013) (PaO_2 = 50.95 mm Hg). The differences in arterial oxygen values between these studies were possibly due to increased body mass in supplementary-fed compared to free-ranging animals, differences in butorphanol to etorphine ratios administered IV or included in the dart, and reduced exertion in the rhinoceros as they were darted from a vehicle rather than a helicopter. This study also found that blood oxygen values (PaO_2 and SaO_2) were marginally improved in immobilized rhinoceros placed in sternal compared to lateral recumbency due to an apparent improvement in pulmonary ventilation (Boardman *et al.* 2014).

Haw *et al.* (2014, 2015) reported on the effects of butorphanol IV in etorphine-azaperone immobilized rhinoceros either habituated to captivity or free-ranging. Both groups of animals were administered similar dosages of both immobilizing drugs and butorphanol. Captive rhinoceros developed severe hypoxaemia (PaO_2 = 27 mm Hg), hypercapnia ($PaCO_2$ = 82 mm Hg) and acidosis (pH = 7.26) within 20 min of darting. The authors suggested that the increased $PaCO_2$ indicated the hypoxaemia was predominantly due to hypoventilation. Other proposed factors contributing to the decrease in PaO_2 included ventilation-perfusion mismatch, intrapulmonary shunts and increased alveolar dead space ventilation associated with recumbency. However, the primary cause of respiratory depression was believed to be due to opioid-induced respiratory depression. The administration of butorphanol IV improved both oxygen and carbon dioxide blood gas values suggesting an improvement in ventilation, although the animal remained hypoxaemic (PaO_2 = 60 mm Hg) and hypercapnic ($PaCO_2$ = 67 mm Hg) (Haw *et al.* 2014). In immobilized free-ranging rhinoceros, initial PaO_2 (35.4 mm Hg) values were similar to the captive animals; however, $PaCO_2$ (63 mm Hg) was not as elevated and yet the animals were more acidaemic (pH = 7.10). These results suggested that field-immobilized rhinoceros ventilated better than did their boma-immobilized counterparts, but developed a metabolic acidosis due to increased activity associated with helicopter darting. It was also hypothesized that an increase in psychological and physiological stress associated with this type of capture resulted in decreased diffusion of oxygen from ventilated alveoli to

perfusing blood due to sympathetic-induced pulmonary hypertension, pulmonary oedema and congestion (Haw *et al.* 2015).

1.3 Cardiovascular system

The primary function of the cardiovascular system is the transport of metabolic substrates required by tissues and the removal of metabolic waste products. It is essential that the various organ systems receive sufficient oxygen, glucose, amino acids, fatty acids, water, essential electrolytes, and various hormones to meet metabolic demands. The by-products of cellular activity, including carbon dioxide, lactic acid and nitrogenous wastes, have to be removed, metabolized, recycled and in some cases excreted from the body. The system must be able to both continuously meet homeostatic requirements of all tissues and adapt rapidly to changes in organ perfusion requirements in times of increased metabolic activity (Stephenson 2007).

1.3.1 Cardiovascular system control

Perfusion pressure, difference between the mean aortic blood pressure and venous caval blood pressure, is the driving force which circulates blood through the systemic circulation. As central venous pressure in the vena cava near the right atrium of the heart is low (3-8 mm Hg), the mean arterial blood pressure and mean aortic blood pressure are often considered to be equivalent (Stephenson 2007). Mean aortic blood pressure is determined by cardiac output and total peripheral resistance (Guyenet 2006). Cardiac output is made-up of two components; stroke volume (blood volume ejected by the left ventricle each cardiac cycle) and heart rate (number of cardiac cycles per minute) (Muir 2007). Stroke volume is the difference between ventricular end-diastolic volume and ventricular end-systolic volume (Stephenson 2007).

Ventricular end-diastolic volume is influenced by ventricular preload, duration of diastole and ventricular compliance. The amount of ventricular filling that occurs during diastole determines preload or pressure within the ventricle, and depends

on venous return to the heart. Cardiac venous return is influenced by factors such as compression of veins due to muscle activity, the pressure differential between the thorax and abdomen associated with respiration, and sympathetic constriction of large veins. These factors facilitate the return of blood to the heart within the venous system (Guyenet 2006; Stephenson 2007).

The duration of diastole will also influence the degree of ventricular filling. The ventricles relax and fill with venous blood during diastole; however, with increasing heart rates, the diastolic period is reduced, limiting the time available for ventricular filling. In humans, at heart rates higher than 160 beats / min, there is insufficient time for complete ventricular filling and a reduction in stroke volume, cardiac output and mean aortic blood pressure results. To ensure sufficient time for ventricular filling during periods of increased metabolic demands, the ventricles take less time to contract and contract with greater force due to increased sympathetic nervous system activity. The sympathetic system both innervates individual myocardial cells and the sino-atrial and atrial-ventricular nodes, and releases adrenaline and noradrenaline from the adrenal medulla into circulation. These catecholamines and noradrenaline released from sympathetic neurons act on myocardial beta $(\beta)_1$-adrenergic receptors with positive chronotropic, inotropic, dromotropic and lusitropic outcomes. The net effect is the maintenance of diastole to allow for sufficient ventricular filling. Ventricular compliance is the ease with which the ventricle walls stretch to accommodate the preload, and a healthy heart is able to accommodate the normal end-diastolic ventricular volume (Stephenson 2007).

Ventricular-end systolic volume depends on the contractility of the ventricles, and an increase in contractility results in an increased stroke volume for any end-diastolic volume. As discussed above, sympathetic nerve activity increases ventricular contractility (Stephenson 2007).

Total peripheral resistance, a component of mean arterial pressure, is determined by vascular resistance to blood flow of which vessel radius, especially of

arterioles, is the primary moderator. Arterioles contain smooth muscle, and by contraction or relaxation, alter resistance to blood flow within the systemic system. Vasoconstriction increases resistance and decreases blood flow and vasodilation has the opposite effect (Stephenson 2007).

Arteriolar resistance in controlled by two mechanisms; intrinsic mechanisms which are metabolic in origin and extrinsic mechanisms which are nerve and hormone dependent (Janssen & Smits 2002). The intrinsic mechanism dominates over the extrinsic mechanism in tissue critical to an animal's survival (myocardium, brain and exercising skeletal muscle) (Stephenson 2007). Cellular metabolic products, such as potassium, carbon dioxide, adenosine and lactic acid, cause arteriolar smooth muscle to relax resulting in vasodilation with increased perfusion of metabolizing tissue cells (Muir 2007). This increased blood flow removes metabolic products, and delivers oxygen and metabolic substrates (Stephenson 2007).

The neurohumoral or extrinsic mechanism includes the autonomic (sympathetic and parasympathetic) nervous system, and adrenaline and noradrenaline released from the adrenal medulla. The autonomic nervous system tends to produce cardiovascular responses which can be large in magnitude but generally of short duration. The release of adrenaline and noradrenaline into circulation by the adrenal medulla has effects complimentary to those of sympathetic nervous stimulation but of longer duration (Muir 2007). The neurohumoral mechanism primarily controls blood flow to non-critical organs, and influences heart rate and stroke volume in regulating cardiac output.

Noradrenaline released from sympathetic neurons, and circulating adrenaline and noradrenaline released from the adrenal medulla, interact with adrenergic receptors differentially distributed in the various organs. Alpha$_1$- and α_2–adrenergic receptors occur on arterioles of all body organs and abdominal veins (Stephenson 2007). Alpha$_2$-adrenergic effects predominate in veins compared to arteries (Muir 2007). Alpha$_{1A}$- and α_{1D}-adrenergic receptors are the prime

mediators of sympathetic arterial smooth muscle contraction (Janssen & Smits 2002). The major effect is to increase total peripheral resistance and maintain perfusion pressure to non-vasoconstricted organs (Stephenson 2007). The constriction of abdominal veins assists with venous blood return to the heart to increase ventricular preload.

Catecholamine stimulation of β_1-receptors located on myocardial cells increases the rate and force of ventricular contraction, increasing heart rate, stroke volume and cardiac output (Janssen & Smits 2002). $Beta_2$-adrenergic receptors in blood vessels are not innervated by the sympathetic nervous system, but rather respond to circulating catecholamines (Muir 2007). Adrenaline and noradrenaline activate β_2-receptors on coronary and skeletal muscle arterioles increasing blood flow in both these tissues. $Beta_2$-vasodilation takes preference over α-vasoconstriction (Stephenson 2007). The parasympathetic nervous system decreases heart rate and cardiac output through stimulation of M_2-receptors located primarily in the sino-atrial and atrio-ventricular nodes, and atria (Janssen & Smits 2002). This system also inhibits the release of noradrenaline from sympathetic neurons innervating the ventricles (Stephenson 2007). Stimulation of the parasympathetic nervous system has minimal effects on most peripheral blood vessels (Muir 2007).

Central cardiovascular control is integrated between multiple regions. Hypothalamic centres modulate cardiovascular responses in the fight-or-flight response, with body temperature changes, and during exercise. The cerebral cortex influences cardiovascular responses to exercise, emotion, ischaemia and hypoxia (Muir 2007). The nucleus tractus solitarius in the medulla is a principle centre for circulatory control and receives input from arterial baroreceptors, volume receptors and peripheral chemoreceptors. The arterial baroreceptors are the afferent component of the baroreflex (Guyenet 2006).

The baroreceptor reflex responds quickly and powerfully to counteract sudden changes in blood pressure. This reflex is responsible for maintaining moment-to-moment stability in blood pressure by adapting cardiac output and vascular

resistance (Janssen & Smits 2002). Baroreceptors located in the walls of the carotid arteries and aortic arch sense changes in distension of the arterial walls due to fluctuations in blood pressure. The receptors send afferent impulses to the CNS via the vagus, carotid sinus and glossopharyngeal nerves, which reflexively alter cardiac output and vascular resistance (in non-critical organs) to maintain mean aortic blood pressure (Charkoudian *et al.* 2005; Stephenson 2007). Decreased distension of arterial walls due to falling blood pressure will decrease afferent baroreceptor activity (Guyenet 2006). In response, the CNS activates sympathetic activity, which increases cardiac output and causes vasoconstriction of arterioles, particularly in non-critical organs (kidney, splanchnic organs and resting skeletal muscle), and increasing total peripheral resistance. The cardiac sympathetic effects are augmented by a decrease in parasympathetic activity. The net result is an increase in blood pressure. The baroreceptor reflex response is reversed for an increase in blood pressure (Stephenson 2007). Baroreceptors become inoperative below 60 mm Hg, and above this pressure, afferent nerve activity progressively increases reaching a maximum at approximately 180 mm Hg (Muir 2007). The activation of peripheral chemoreceptors by hypoxia and hypercapnia increases sympathetic nerve activity to the heart and blood vessels (sympathetic chemoreflex) resulting in increases in blood pressure and heart rate. It is possible that central chemoreceptors also contribute to this response (Guyenet 2006).

In cases of psychogenic stress resulting in the "fear, fight or flight" response, there is increased sympathetic and decreased parasympathetic activity, which will result in an increase in blood pressure due to increased heart rate, stroke volume, cardiac output and total peripheral resistance associated with vasoconstriction in non-critical organs. In these cases the baroreceptor reflex is reset so that blood pressure is regulated at an elevated level rather than trying to correct the increase in blood pressure (Stephenson 2007).

1.3.2 Cardiovascular effects of immobilizing drugs

1.3.2.1 Opioids

Cardiovascular effects of endogenous or exogenous opioids are complex and can be difficult to define as they are mediated by receptors located centrally within the brain and peripherally by tissue-associated opioid receptors. Activity of opioid receptors may be modulated indirectly by other G protein-coupled receptors, and some opioid effects are mediated by receptor-independent mechanisms (Headrick, Pepe & Peart 2012). Opioid effects on the heart and vasculature can be due to interaction with receptors or direct interaction with myocardial cells or blood vessel smooth muscle (Pugsley 2004). Drug effects are further complicated by changes in opioid receptor expression due to alterations in physiological states (such as hypotension) and disease (such as hypertension) (Pugsley 2002).

Cardiovascular effects of centrally administered exogenous opioids are variable depending on species, dose, opioid analog receptor specificity, and the route and site of administration (Feuerstein 1985; Feuerstein & Sirén 1987). Generally, opioids injected into the lateral cerebral ventricle result in hypertension and tachycardia; however, hypotension and bradycardia are observed following administration into the cisterna magna or the fourth cerebral ventricle (Feuerstein & Sirén 1988). Responses vary depending on the exact location of the injection site and type of opioid administered (Sun *et al.* 1996). Microinjection of various opioids into hypothalmic nuclei located less than 1 mm apart can result in both pressor and depressor effects (Feuerstein & Sirén 1988). Outcomes of intrahypothalamic injections may also vary with dose of opioid administered; increases in blood pressure and heart rate were observed with low doses of a MOR specific agonist and the reverse occurred with higher doses (Feuerstein & Sirén 1988). Injection of endomorphins, with a high selectivity for MOR, into the subarachnoid space and central spinal fluid (CSF) of the spinal cord in rats elicited a decrease in systemic blood pressure and heart rate (Feng *et al.* 2012). Central opioid cardiovascular outcomes can be further complicated by concurrent

changes in the respiratory system induced by the administration of this group of drugs. Controlling respiration through artificial ventilation can alter a cardiovascular depressor response to a pressor response following central encephalin analog administration in rats (Feuerstein 1985).

Opioid receptors within both the sympathetic and parasympathetic nervous systems appear to be involved in cardiovascular modulation (Feuerstein & Sirén 1987). The outcome of giving an opioidergic drug is influenced by the balance between these two components of the autonomic system at the time of administration (Headrick *et al.* 2012). Pressor effects and tachycardia due to specific MOR- and DOR-agonists in conscious rats is most likely due to activation of the sympathoadrenomedullary axis (Feuerstein & Sirén 1987). Morphine-induced bradycardia, hypotension, reduced inotropy and cardiac output in rats, rabbits, and dogs can be blocked by severing the vagus nerve (Headrick *et al.* 2012). Blocking of opioid peptide receptors restores reduced heart rate and blood pressure due to acute vagal stimulation in dogs (Headrick *et al.* 2012).

MOR appear to be absent in the heart, although may be present on cardiac nerves or on cardiac vascular endothelial cells. Both KOR and DOR are found in the heart, with DOR in large numbers (Pugsley 2004). Distribution of receptor types varies within the heart between ventricles and atria; for example, both ventricular and atrial myocytes have DOR and KOR, although KOR binding occurs more in the right atrium compared to the left, and DOR appear to be concentrated in the atria and sino-atrial node (Feuerstein & Sirén 1987; Headrick *et al.* 2012; Pugsley 2004). KOR and DOR agonists have been shown to inhibit atrial but not ventricular activity, whereas MOR agonists reduce ventricular contractility without altering atrial function (Pugsley 2002).

Negative opioid inotropic effects can either be opioid peptide receptor dependent or independent (Headrick *et al.* 2012). Positive opioid inotropic effects have also been observed and it is hypothesized that activation of DOR on prejunctional cardiac vagal nerves or parasympathetic ganglia reduces the release of

acetylcholine (Headrick *et al.* 2012; Pugsley 2002). MOR activation may decrease the rate of electrical impulses and sinoatrial and atrioventricular nodal function in the heart with a negative chronotropic outcome. By comparison, MOR and DOR stimulation can increase heart rate due to inhibition of vagal activity and baroreflex-mediated bradycardia. These effects on heart rate may be mediated by both central and peripheral modes of action (Headrick *et al.* 2012). In rats, it has been shown that central MOR activation, especially of the nucleus tractus solitarius, markedly impairs the baroreceptor control of sympathetic and cardiovascular function. Suppression of the baroreceptor reflex by stimulation of central opioid receptors is increased with low reflex activity and can be overcome as baroreceptor information reaching the brain increases (Gordon 1986; Gordon 1990).

Studies suggest that opioids can have receptor independent actions on cardiac myocytes by blocking Na^+, possibly K^+ and potentially L-type Ca^{2+} channels influencing repolarization or depolarization of these cells (Pugsley 2002). Activity of opioid agonists and antagonists is relatively receptor specific at low concentrations, yet at high concentrations their activity becomes receptor independent (Pugsley 2002, Pugsley 2004).

Endogenous enkephalins inhibit, or reverse the chronotropic and inotropic effects of β-adrenoceptor activity. This inhibition of catecholamine effects can occur directly on the cardiac myocytes and does not require neural involvement (Headrick *et al.* 2012). It has been shown in single isolate rat ventricular myocytes that opioid peptide stimulation at DOR modulates β-adrenergic responses and may be important in regulating myocardial responses to stress (Xiao *et al.* 1997).

Opioid effects on vascular smooth muscle are variable depending on species and between different vascular beds. In rats, contraction of the aorta, dilation of mesenteric arterioles, and no effect at muscular venules have been observed (Feuerstein & Siren 1987). These effects appear to be direct, involving various opioid receptor sub-types on smooth muscle or may be indirect (Feuerstein &

Sirén 1987). Vasodilation in humans and dogs following morphine administration is histamine-modulated (Headrick *et al.* 2012; Robertson, Muir & Sams 1981). Various studies indicate that the different pressor responses due to opioids depend not only on the opioid receptor targeted but also the dose of opioid given (Headrick *et al.* 2012).

Etorphine

Opioids at therapeutic analgesic doses have minimal cardiovascular effects in domestic animals; however, these may become more pronounced when administered at supra-therapeutic doses or when potent opioids are used such as in the immobilization of wildlife (Kukanich & Papich 2009; Leigh & Mathews 2007). Cardiovascular responses to etorphine differ between species. In rats, dogs, cats, and monkeys, etorphine causes bradycardia and hypotension, although tachycardia and increased blood pressure has been reported in elephants (Daniel & Ling 1972; Haigh 1990). In artiodactyls, blood pressure tends to decrease with etorphine administration; bradycardia may occur especially at higher doses (Haigh 1990). Etorphine and carfentanil, are reported to cause a prolonged hypertension and bradycardia in goats (Heard *et al.* 1996)

Increased cardiac output, total peripheral resistance and arterial blood pressure are consistent findings in opioid-immobilized perissodactyls including domestic and Mongolian horses, ponies, Grevy's zebra, and white rhinoceros (Haigh 1982; Haigh 1990; Heard *et al.* 1992; Lees & Hillidge 1975). Horses, administered etorphine remained tachycardic and hypertensive for 30 min (Bogan, MacKenzie & Snow 1978), with tachycardia present over a wide range of etorphine doses (with acepromazine) (Daniel & Ling 1972). Similarly, the same drug combination also caused a pronounced hypertension, of variable duration, in other horses (Lees & Hillidge 1975; Schlarmann *et al.* 1973). Increased sympathetic activity, due to direct and, or, indirect stimulation, is proposed as the cause of these cardiovascular changes with etorphine (Bogan *et al.* 1978; Heard *et al.* 1996; Lees & Hillidge 1975; Schlarmann *et al.* 1973). Indirect stimulants may include hypoxia and, or, hypercapnia due to impaired respiration, stress associated with

research procedures, pain or a combination of factors (Heard *et al.* 1996; Lees & Hillidge 1975). Increased sympathetic activity may lead to direct myocardial stimulation, terminal arterial and arteriolar constriction, and, or, adrenal gland catecholamine release (Bogan *et al.* 1978; Heard *et al.* 1996; Lees & Hillidge 1975; Schlarmann *et al.* 1973). This increased central sympathetic outflow has also been suggested as the cause of increased arterial pressure observed with intravenous administration of morphine in humans (Carter, Sauder & Ray 2002).

Butorphanol

Butorphanol is a potent analgesic providing mild sedation with a rapid onset of action and minimal adverse effects. In horses, it is commonly used to relieve moderate to severe pain (Orsini 1988; Wenger *et al.* 2007). Butorphanol causes minimal cardiovascular effects in humans and dogs (Robertson *et al.* 1981). Although butorphanol IV does not alter heart rate in human subjects, there are alterations of other indices of cardiovascular function, such as increases in cardiac and stroke volume index, left ventricular end diastolic pressure, and pulmonary arterial pressure. These changes suggest that butorphanol may increase cardiac workload (Gillis *et al.* 1995). In contrast, Plumb (2008) suggested that butorphanol may decrease heart rate secondary to an increase in parasympathetic tone, along with mild decreases in arterial blood pressure. In humans, butorphanol attenuates the baroreceptor receptor reflex control of heart rate in response to an increase in blood pressure, but not with decreased blood pressure. Possible explanations include hypercapnia associated with butorphanol administration altering baroreflex sensitivity to increased blood pressure, or central effects at the nucleus tractus solitaries which is the primary termination for baroreceptor afferent fibres (Wajima, Inque & Ogawa 1993).

In awake dogs, butorphanol (0.1 and 0.4 mg/kg) IV was reported to cause small but significant decreases in heart rate and mean arterial blood pressure, possibly due to a peripheral vasodilation (Trim 1983). Similar results have been reported in halothane-anaesthetized dogs administered butorphanol (0.2 mg/kg) IV where significant, although transient (< 15 min.) decreases in heart rate, mean and

diastolic arterial blood pressure occurred. However, cardiac index, stoke volume, pulmonary artery wedge pressure and systemic vascular resistance did not change significantly (Greene, Hartsfield & Tyner 1990). In alpacas, butorphanol causes a mild decrease in systemic vascular resistance but no significant changes in other cardiovascular variables (Pereira *et al.* 2005).

Intravenous butorphanol (0.1-0.4 mg/kg) in horses at rest did not significantly change heart rate, mean and diastolic blood pressures or cardiac output (Robertson *et al.* 1981). However, in separate studies, 0.22 mg/kg and 0.1 mg/kg butorphanol IV to ponies and horses, respectively, resulted in increased heart rate which persisted for up to two hours in the horses. There were significant differences in the magnitude of change between individuals. It has been suggested that these cardiac effects were due to CNS stimulation (Knych *et al.* 2012).

Cardiovascular effects of butorphanol in equids appear to be dose-related. In halothane-anaesthetized ponies and horses, low doses of butorphanol (0.022 to 0.05 mg/kg) did not produce changes in mean arterial blood pressure or heart rate; however, a larger dose (0.2 mg/kg) induced hypotension (Hofmeister, Mackey & Trim 2008). In horses sedated with detomidine, the addition of butorphanol reversed the increased systemic vascular resistance caused by α_2-agonist. Other cardiovascular changes, including decreased heart rate and cardiac output, and increased systemic arterial pressure, were unaltered (Nyman *et al.* 2009)

1.3.2.2 Butyrophenones

Butyrophenones are antagonists at multiple receptors with the strongest affinity for D-receptors and weaker binding at adrenergic α_1-adrenergic, 5-HT-, M_3- and H_1- receptors (Lemke 2007). Clinically, butyrophenones are generally reported to increase heart rate, decrease contractility and slightly decrease blood pressure, with no change or an increase in cardiac output (Muir 2007). However, cardiovascular outcomes are variable due to multiple receptor affinities. D_1-like and D_2-like receptors exist in the cardiovascular system; however, there is limited understanding of their functions (Missale *et al.* 1998). In blood vessels, activation

of D_2-like receptors indirectly induces vasodilation by inhibiting noradrenaline release on postganglionic sympathetic nerve terminals and activation of D_1-like receptors on vascular smooth muscle directly results in vasodilation primarily in the renal, mesenteric, cerebral and coronary circulations (McGrath & Wang 1994; Missale *et al.* 1998). In the adrenal medulla, D_1-like receptor activation stimulates and D_2-like receptor activation inhibits the release of catecholamines. D_2-like receptor activations in peripheral sympathetic ganglia and nerve endings inhibit the release of noradrenaline (Missale *et al.* 1998). Both D_1- and D_2-like receptors including D_1-, D_2-, D_4- and D_5-receptors, occur in the epicardium, myocardium and endocardium of human atria and ventricles with variable distribution (Cavallotti *et al.* 2010). The administration of exogenous dopamine increases cardiac contractility (Pivonello *et al.* 2007).

Alpha$_1$-adrenergic receptors compared to α_2-adrenergic receptors, are the predominant α-receptor located on vascular smooth muscles. Activation of these receptors result in smooth muscle contraction which is more pronounced in arterial resistance vessels (small arteries and arterioles) compared to veins and results in increased peripheral resistance (Klabunde 2013). 5-HT can have profound and varied effects in the cardiovascular system: centrally 5-HT can inhibit noradrenaline release and sympathetic activity. 5-HT-receptors on cardiomyocytes have positive chronotropic and inotropic effects, and, depending on the species, effects on blood vessels can result in both vasoconstriction and vasodilation (Ni & Watts 2006). M_3-acetylcholine receptors in the heart play a role in the parasympathetic control of cardiac function including regulation of heart rate and cardiac repolarization, and modulation of inotropic effects (Wang *et al.* 2007). In vascular tissue, the activation of H_1- and H_2-receptors results in vasoconstriction and vasodilation, respectively; however in humans, vasodilation is the predominant effect (Ebeigbe & Talabi 2014). Simultaneous stimulation of these two histamine receptors in the heart results in positive inotropic and chronotropic effects, a negative dromotropic effect and increased automaticity (Krzan 1996).

Azaperone

Azaperone, a D-receptor antagonist, is administered primarily as a tranquillizer in domestic animals and wildlife species; however, due to its blocking activity at peripheral α_1-receptors, it is also used to moderate opioid-induced hypertension in immobilized megaherbivores (Burroughs *et al.* 2012a). In horses tranquillized with azaperone (0.4 and 0.8 mg/kg), a 30% reduction in mean arterial blood pressure, due to reduction in total peripheral resistance, developed for up to four hours. A proposed mechanism for this drop in blood pressure was the blockade of α-receptors and reduction in motor tone. The higher dose of azaperone (0.8 mg/kg) resulted in a small increase in heart rate due to possible baroreceptor stimulation in response to the hypotension (Lees & Serrano 1976; Serrano & Lees 1976). Decreases in heart rate and cardiac output were reported in young pigs administered 2.5 mg/kg azaperone intramuscularly, and a decrease in arterial pressure at a dose of 0.3 to 3.5 mg/kg in adult pigs (Lemke 2007). Van Woerkens *et al.* (1990) found that azaperone (5 mg/kg IM) in conscious pigs reduced cardiac output by 10% and mean arterial pressure by 35%. The change in cardiac output was due to a decrease in stroke volume rather than heart rate. Blood flow to the brain was maintained despite the reduction in mean arterial blood pressure due to cerebral vasodilation (Van Woerkens *et al.* 1990). In addition to a pronounced α-adrenergic blocking effect, azaperone appears to reduce β_2-antagonist action and retard sympathetic reflexes in pigs (Gregory & Wilkins 1986).

1.3.3 Cardiovascular effects of immobilizing drugs in white rhinoceros

Published reports on the cardiovascular effects of immobilizing drugs in white rhinoceros are limited. An early case report of a captive white rhinoceros (2000 kg) immobilized with etorphine (2.8 mg) found initial systolic and diastolic blood pressures of 300 and 250 mm Hg, which gradually decreased and stabilized at 240 and 200 mm Hg, respectively, for the remainder of a 120 minute procedure. Heart rate remained between 80 to 100 beats per minute (LeBlanc *et al.* 1987). This animal was hypertensive and tachycardic compared to mean blood pressures (systolic 160 mm Hg, diastolic 104 mm Hg) and mean heart rate (39 beats per minute) in captive conscious white rhinoceros standing at rest (Citino & Bush

2007). Increased sympathetic activity was proposed as the cause of the pressure effects in the immobilized rhinoceros (LeBlanc *et al.* 1987). Heard *et al.* (1992) reported more moderate increases in arterial blood pressure and heart rate in a 28 year old captive female white rhinoceros immobilized with an initial lower etorphine dose, with repeated doses over 210 min. Opioid-induced hypercapnia, hypoxaemia and pain associated with reproductive manipulation were suggested as contributing causes to the hypertension (Heard *et al.* 1992). Portas (2004) also suggested that etorphine-induced hypertension, secondary to increased cardiac output and peripheral resistance, is a common occurrence in white rhinoceros, and may be related to increased sympathetic activity. This may have contributed to the marked tachycardia (152 beats per minute) also reported for free-ranging white rhinoceros immobilized with etorphine only (Kock *et al.* 1995).

When etorphine was combined with azaperone to immobilize free-ranging rhinoceros, a lower mean arterial blood pressure (141 mm Hg) was measured compared to that in animals that received etorphine plus fentanyl (183 mm Hg) (Hattingh *et al.* 1994). It was hypothesized that the difference in blood pressure between treatments was due to the peripheral α_1-adrenergic receptor properties of azaperone (Hattingh *et al.* 1994). Bush *et al.* (2004) found that free-ranging rhinoceros immobilized with a combination of etorphine and azaperone (ratio ± 1:10-20, in mg) were hypertensive (systolic blood pressure 164 mm Hg, normal resting value 124 mm Hg (Citino & Bush 2007)) and tachycardic (107 beats per minute). However, compared to standing resting values for white rhinoceros, which were not available at the time, the immobilized rhinoceros were only mildly hypertensive, although heart rate was significantly elevated (Bush *et al.* 2004, Citino & Bush 2007). The provision of supplementary oxygen did not alter blood pressure or heart rate in these animals (Bush *et al.* 2004).

In free-ranging rhinoceros immobilized with etorphine and azaperone, with butorphanol included in the dart or administered IV once the animals became immobilized, the initial tachycardia observed in both treatments decreased over time. It was suggested that the reduction in heart rate was due to butorphanol,

although possible mechanisms for this effect were not discussed (Miller *et al.* 2013). Boardman *et al.* (2014) also reported a significant decrease in heart rate (118 to 74 beats per minute) within 10 min after administration of butorphanol (10 x etorphine dose in mg) IV in white rhinoceros immobilized with etorphine and azaperone. Systolic and diastolic blood pressures significantly declined (systolic 162 to 139 mm Hg, diastolic 104 to 80 mm Hg). The changes in blood pressure were attributed to a prolonged onset of azaperone effects compared to those of etorphine (Boardman *et al.* 2014). Similarly, Haw *et al.* (2015) found that butorphanol (15 x etorphine dose in mg) IV and provision of supplementary oxygen resulted in significant decreases in heart rate (136 to 99 beats per min.) and mean arterial blood pressure (158 to 114 mm Hg) within four min of administration. There were no further changes in cardiovascular parameters for the remaining 14 min of the trial, and the animals remained tachycardic and normotensive (Haw *et al.* 2015).

1.4 Summary and thesis aims

Chemical immobilization is an essential procedure used in the management of free-ranging white rhinoceros including moving animals between isolated populations, re-introductions into previous ranges, disease investigation, anti-poaching measures and treatment of injuries. Anaesthesia and sedation are also important procedures used in the husbandry and welfare of captive individuals. At present, white rhinoceros can only be immobilized with a potent opioid combined with a tranquillizer or sedative, and etorphine plus azaperone is most commonly used (Burroughs *et al.* 2012b).

Etorphine is the immobilizing opioid of choice for white rhinoceros as it is sufficiently potent to be included in a dart, results in rapid induction and is commercially available. Azaperone is included in etorphine-darts as it reduces induction time (dart in to immobilization) and limits opioid-induced hypertension in immobilized white rhinoceros. However, this drug combination results in changes to respiratory and cardiovascular clinical parameters which indicate

significant physiological alterations in these two systems and may be associated with mortalities (Van Zijll Langhout *et al.* 2016; Kock *et al.* 1995).

Various procedures are used to limit the cardiorespiratory physiological alterations associated with etorphine-azaperone immobilization, which include minimizing immobilization duration, use of partial opioid antagonists (nalorphine and diprenorphine) and respiratory stimulants (doxapram), and nasal or tracheal oxygen insufflation (Buss *et al.* 2015). Butorphanol, a mixed opioid agonist-antagonist, has in recent years been evaluated as a possible antagonist to respiratory depression in immobilized rhinoceros (Boardman *et al.* 2014; Haw *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013; Wenger *et al.* 2007). Anecdotal field evidence suggested that butorphanol improved respiratory function; however, study results are equivocal suggesting that either improved ventilation or metabolic shifts result in improved arterial partial pressure of oxygen, carbon dioxide or both (Boardman *et al.* 2014; Haw *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013; Wenger *et al.* 2007). Personal field observations suggested that etorphine-azaperone immobilization could be maintained in rhinoceros for longer periods of time and an initial tachycardia decreased rapidly if butorphanol was administered intravenously.

The adverse respiratory parameters in immobilized white rhinoceros are well documented in scientific literature (Boardman *et al.* 2014; Haw *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013; Wenger *et al.* 2007). Cardiovascular changes are less well recorded (Hattingh *et al.* 1994; Heard *et al.* 1992; LeBlanc *et al.* 1987; Raath 1999; Waltzer *et al.* 2000). Similarly, studies which investigate the origins of drug-specific physiological effects that influence the clinical parameters recorded are limited. The reason for a paucity of these types of studies is multifactorial (Haw *et al.* 2014 & 2015; Miller *et al.* 2013). In free-ranging rhinoceros darted from helicopters, the physiological drug effects are overlain by multiple other influences, including variable environmental factors (temperature and humidity), an adrenergic stress response and increased metabolic activity prior to immobilization, limited health status evaluation and drug dose rates based

on an estimated body mass. In both free-ranging and captive rhinoceros, monitoring of respiratory and cardiovascular variables starts only once the animal is sufficiently immobilized to be safely approached. By this time, both etorphine and azaperone are influencing physiological responses, centrally and peripherally, and it is not possible to differentiate their individual specific effects.

Unraveling the respiratory and cardiovascular physiological drug effects of etorphine, azaperone and butorphanol is further complicated by the complexity of these two integrated biological systems. Enormous strides have been made in understanding the physiology of these systems, however, our understanding is incomplete and in some aspects, contradictory. Mathematical models based on current knowledge are used to best describe how respiratory rhythm is generated and modulated in the ventral respiratory column (Rekling & Feldman 1998; Smith *et al.* 2000). Coordinated motor neuron outputs arising centrally are generated to control the various muscle groups involved in breathing including those of the thorax, upper respiratory tract and the diaphragm. Modulation of the system is primarily due to carbon dioxide levels which are sensed by multiple chemosensing areas located within the brain stem making up the central chemoreceptor (Haji et al. 2000; Pattinson 2008). Peripheral chemoreceptors in carotid bodies play a supporting role which becomes more important with developing hypoxia. However, the mechanisms underlying chemosensitivity are not fully understood (Feldman *et al.* 2013). Modulation can also be conscious with inputs from the brain cortex. The mechanisms that generate and control respiration have to be sufficiently flexible and integrated to adjust rapidly to changes in metabolic demand, accommodate chronically altered conditions such as disease or persistent hypoxia, and allow for short-term perturbations such as postural changes, swallowing and vocalization (Bianchi *et al.* 1995; Mitchell & Johnson 2003; Smith *et al.* 2007).

The cardiovascular system is similar to the respiratory system in that it responds rapidly to changing circumstances including increases in metabolic requirements to ensure sufficient metabolic substrates are supplied to active tissues and waste

products removed. The control of this system is complex and depends on two principle mechanisms; intrinsic control which is metabolic in origin, and extrinsic control, which is nerve and hormone dependent (Janssen & Smits 2002). Central cardiovascular control is integrated between multiple regions and receives input from baroreceptors and volume receptors within the vascular system. Peripheral chemoreceptors also contribute to cardiovascular control (Guyenet 2006). The system is also modulated by regions of the CNS which respond to changes in body temperature, exercise, emotion, ischemia and hypoxia (Muir 2007). During the "flight and fight" response, neurohumoral mechanisms primarily influence heart rate, stroke volume and blood flow to non-critical organs through the autonomic nervous system and release of catecholamines from the adrenal medulla (Stephenson 2007). Outcomes are dependent on direct autonomic innervation of tissues, and the type and distribution of both adrenergic and cholinergic receptors within the cardiovascular system.

The etorphine-azaperone immobilizing combination can influence the respiratory and cardiovascular systems both centrally and peripherally by both receptor and non-receptor associated mechanisms. Etorphine is a pure agonist at MOR, DOR and KOR receptors and its respiratory and cardiovascular effects will depend on the distribution of opioid receptors within these systems and the cellular functions they influence. Current understanding of opioid effects are clouded, in part, by pharmacological evidence which suggests the existence of a number of opioid receptor-subtypes; however molecular evidence does not support the notion of opioid receptor-subtypes (Dietis *et al.* 2011; Feng *et al.* 2012). Opioids influence the respiratory and cardiovascular systems both centrally and peripherally, activate various intracellular signal pathways, and can influence the activity of receptors other than opioid receptors (Boom *et al.* 2012; Koo & Eikermann 2011). The principle respiratory effect of opioids is a profound depression of neuronal rhythm generation and respiratory response to increasing arterial carbon dioxide levels; the peripheral chemoreceptor hypoxic response is also inhibited (McDonald & Lambert 2005; Pattinson 2008). Current understanding of opioid respiratory effects has resulted principally from extensive studies on human

therapeutic opioid use and research done using rodent or domestic animal models. However, it is not known to what extent this current understanding applies to etorphine use in immobilizing rhinoceros.

The physiological basis of opioid-induced cardiovascular distresses appears to be less well understood when compared to those of the respiratory system. Central opioid influences are variable and outcomes can depend on the exact administration location, and receptor activity, affinity and dose of opioid being studied (Feuerstein & Sirén 1988). Peripherally, opioid receptors are found in both parasympathetic and sympathetic nerves involved in cardiovascular control. In the heart, the distribution of opioid receptor types differs between ventricle and atria, and variable chronotropic and inotropic outcomes have been observed depending on opioid study methodologies (Feuerstein & Sirén 1987; Headrick *et al.* 2012; Pugsley 2004). Opioids can also impair baroreceptor control of sympathetic and cardiovascular function (Gordon 1986; Gordon 1990). Opioids may alter cardiac function by receptor independent actions and possibly influence the catecholamine response at myocyte β-adrenergic receptors (Pugsley 2002; Xiao *et al.* 1997).

Respiratory and cardiovascular physiological influences of azaperone are poorly understood. It is an antagonist at several receptors (D-, α_1-, 5-HT-, M_3- and H_1-) suggesting that it may potentially have multiple effects on the respiratory and cardiovascular systems. The primary activity of azaperone is the blockade of D-receptors and dopaminergic mechanisms modulate respiration both centrally and peripherally; although the specific effects are not conclusive (Hsiao *et al.* 1989). Similarly, the role of D-receptors in controlling cardiovascular functions is not well understood (Missale *et al.* 1998). However, azaperone is reported to antagonize α_1-adrenergic receptors and decrease peripheral resistance which is believed to moderate hypertension in etorphine-immobilized rhinoceros (Hattingh *et al.* 1994; Klabunde 2013; Lemke 2007).

Butorphanol's physiological effects are possibly the least understood of the drugs used in white rhinoceros immobilization. In humans and domestic animals, butorphanol is administered as the principle opioid for its agonist and analgesic effects; however, in immobilized rhinoceros, it is used to partially antagonize the agonist effects of etorphine. The use of this mixed opioid agonist-antagonist in this manner would appear to be unique in published literature and reports on physiological outcomes are limited (Boardman *et al.* 2014; Haw *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013; Wenger *et al.* 2007). Butorphanol administered on its own tends to depress respiration. Initially it was thought that this depression was limited due to a ceiling effect; however, it has been shown that it can be marked. Reports suggest that its respiratory effects are variable depending on dose, species and presence of other drugs (Vivian *et al.* 1999; WHO Expert Committee on Drug Dependence 2006). In the horse, a domestic perissodactyl used as a physiological equivalent for comparison with rhinoceros, butorphanol's cardiovascular effects are not well defined and variable pressor effects have been documented.

The research objectives and aims of this thesis are:

Objective 1

Determine the effects of etorphine and etorphine plus azaperone on respiration in immobilized white rhinoceros.

Aims:

1.1 Determine the effects of etorphine-azaperone on clinical respiratory parameters in immobilized rhinoceros.

1.2 Determine the effects of etorphine on clinical respiratory parameters in immobilized rhinoceros.

1.3 Evaluate which clinical respiratory parameters can be attributed to etorphine-induced respiratory physiological alterations in immobilized rhinoceros.

Objective 2

Determine through which mechanisms butorphanol alters respiratory depression in etorphine or etorphine plus azaperone immobilized white rhinoceros.

Aims:

2.1 Determine the effects of intravenous butorphanol administration on clinical respiratory parameters in etorphine-azaperone immobilized rhinoceros.

2.2 Determine the effects of intravenous butorphanol administration on clinical respiratory parameters in etorphine-immobilized rhinoceros.

2.3 Evalute which clinical respiratory parameter changes can be attributed to intravenous butorphanol administration in etorphine-immobilized rhinoceros.

Objective 3

Determine the effects of etorphine and etorphine plus azaperone on the cardiovascular system in immobilized white rhinoceros.

Aims:

3.1 Determine the effects of etorphine-azaperone on clinical cardiovascular parameters in immobilized rhinoceros.

3.2 Determine the effects of etorphine on clinical cardiovascular parameters in immobilized rhinoceros.

3.3 Evalute which clinical cardiovascular parameters can be attributed to etorphine-induced cardiovascular physiological alterations.

Objective 4

Determine through which mechanisms butorphanol alters cardiovascular parameters in etorphine and etorphine plus azaperone immobilized white rhinoceros.

Aims:

4.1 Determine the effects of intravenous butorphanol administration on clinical cardiovascular parameters in etorphine-azaperone immobilized rhinoceros.

4.2 Determine the effects of intravenous butorphanol administration on clinical cardiovascular parameters in etorphine-immobilized rhinoceros.

4.3 Evalute which clinical cardiovascular parameter changes can be attributed to intravenous butorphanol administration in etorphine-immobilized rhinoceros.

To reduce confounding variables associated with field capture, all study trials were conducted in wild-caught white rhinoceros adapted to holding facilities, and included animals of known body mass and health status. Trials were also conducted at the same time of day with minimal disturbance to the rhinoceros prior to darting.

CHAPTER 2

Evaluation of cardiorespiratory, blood gas, and lactate values during extended immobilization of white rhinoceros (*Ceratotherium simum*).

Buss, P., Olea-Popelka, F., Meyer, L., Hofmeyr, J., Mathebula, N., Kruger, M., Brüns, A., Martin, L. & Miller, M.

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EVALUATION OF CARDIORESPIRATORY, BLOOD GAS, AND LACTATE VALUES DURING EXTENDED IMMOBILIZATION OF WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*)

Peter Buss, B.V.Sc., M.Med.Vet., Francisco Olea-Popelka, D.V.M., Ph.D., Leith Meyer, B.V.Sc., Ph.D., Jennifer Hofmeyr, B. Tech. (Vet), B.Sc.Hon., Nomkhosi Mathebula, B.Tech. (Vet), Marius Kruger, B.Sc.Hon., M.Sc., Angela Brüns, D.V.M., Laura Martin, D.V.M., and Michele Miller, D.V.M., Ph.D.

Abstract: Ten white rhinoceros (*Ceratotherium simum*) were immobilized for a total of 13 procedures in holding facilities in Kruger National Park using etorphine, azaperone, and hyaluronidase to assess the effect of extended immobilization on serial cardiorespiratory, blood gas, and lactate values. Butorphanol was administered intravenously following initial blood collection and physiologic assessment (t = 0). Respiratory and cardiovascular parameters, body temperature, and arterial blood gases were monitored at 10-min intervals for a total of 100 min. Initial parameters at the time of recumbency revealed severe hypoxemia, hypercapnia, tachycardia, an increased alveolar-arterial (A-a) gradient, and mildly elevated lactate levels. At 10 min and 20 min, there were significant ($P < 0.05$) changes in the following physiologic parameters: heart rate decreased [96 and 80 beats/min, respectively, vs. 120 beats/min], arterial partial pressure of oxygen (PaO_2) increased [48 and 45 mm Hg, respectively vs. 30 mm Hg], arterial hemoglobin oxygen saturation increased [79% and 74%, respectively, vs. 47%], A-a gradient decreased [29.13 and 30.00 mm Hg, respectively, vs. 49.19 mm Hg], and respiratory rate decreased [5 and 5 breaths/min vs. 7 breaths/min]. Blood lactate levels also decreased from 2.54 mM/L to 1.50 and 0.89 mM/L, respectively. Despite initial improvements in blood oxygen levels at t = 10 and 20 min, the rhinoceros remained severely hypoxemic for the remainder of the procedure (median PaO_2 = 50.5 mm Hg, 95% confidence interval, 43.8–58.1). Median values for respiratory rate (5 breaths/min) and arterial partial pressure of carbon dioxide ($PaCO_2$; 68.5 mm Hg) did not change significantly for the remaining 80 min. Median lactate, base excess, bicarbonate, and pH values improved between 20 and 100 min despite the persistent hypercapnia, indicating that the animals adequately compensated for respiratory and lactic acidosis. White rhinoceros were immobilized for 100 min with no negative effects, a desirable outcome if procedures require extended chemical immobilization without oxygen supplementation.

Key words: Blood gas, butorphanol, cardiorespiratory, *Ceratotherium simum*, white rhinoceros.

INTRODUCTION

Free-ranging white rhinoceros (*Ceratotherium simum*) are routinely immobilized with etorphine, a potent opioid, combined with the butyrophenone tranquilizer azaperone for medical and management procedures.[21] Tachycardia, hypopnea, hypoxemia, and hypercapnia with respiratory and metabolic acidosis are well-documented side effects of the drug combination in this species.[2,4,17,28] These potentially fatal complications are managed by minimizing the time an animal is kept immobilized before a complete opioid antagonist is administered.[1] Partial opioid antagonists, including nalorphine and diprenorphine; nasal or tracheal oxygen insufflation; and respiratory stimulants such as doxapram have also been used to counteract these negative effects with limited and variable degrees of success.[1,2,21,24] At times there is a requirement to keep an animal immobilized in the field for a prolonged period of time (≥20 min), such as during the treatment of

From the Veterinary Wildlife Services, South African National Parks, Kruger National Park, Private Bag X402, Skukuza 1350, South Africa (Buss, Hofmeyr, Mathebula, Kruger, Brüns); Colorado State University, College of Veterinary Medicine and Biomedical Science, Department of Clinical Sciences, Fort Collins, Colorado 80523, USA (Olea-Popelka, Martin); Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0002, South Africa (Meyer); and DST/NRF Centre of Excellence for Biomedical Tuberculosis Research, Division of Molecular Biology and Human Genetics, Stellenbosch University, Tygerberg 7505, South Africa (Miller). Present addresses (Mathebula): Veterinary Wildlife Services, South African National Parks, P.O. Box 110040, Hadison Park, Kimberley 8306, South Africa; (Brüns): Research and Scientific Services Department, National Zoological Gardens of South Africa, P.O. Box 754, Pretoria 0001, South Africa. Correspondence should be addressed to Dr. Buss (peter.buss@sanparks.org).

injuries caused by poaching, including the removal of snares and bullet wounds.[28] Frequently these operations are helicopter based and are performed without the advantages of inhalation anesthesia, oxygen insufflation, or mechanical ventilation.[2,28] Etorphine, the primary immobilizing agent, is a μ, κ, and Δ opioid peptide (MOP, KOP, and DOP, respectively) receptor agonist.[31] Butorphanol, a synthetic opioid reported to have KOP receptor agonist and MOP receptor antagonist effects, has anecdotally been used to improve ventilation in opioid-immobilized white rhinoceros.[21,31] The objective of this descriptive study was to record and evaluate the cardiorespiratory, blood gas, and lactate values in white rhinoceros immobilized for an extended time period (100 min) with no oxygen support and a single dose of butorphanol. The lack of a control group in this clinical study prevents the rendering of definitive conclusions about the effects of butorphanol; however, it does raise a number of potential research questions to be pursued in future analytical studies.

MATERIALS AND METHODS

Study area and sample population

The study animals were white rhinoceros captured in Kruger National Park (24°59'44.50"S, 31°35'11.17"E; altitude 317 m), South Africa, and placed into holding facilities for management purposes. The management and immobilization of the rhinoceros were conducted according to the South African Parks Standard Operating Procedures for the Capture, Transportation and Maintenance in Holding Facilities of Wildlife. These animals were part of another study that required a prolonged period of immobilization.

Ten animals were used in this descriptive study; three individuals were immobilized twice on separate occasions. Rhinoceros ranged in age from 3.5 to 15 yr and included four males and six females. Prior to being included in this trial, all animals had adapted to captivity and were eating and defecating normally. Animals were deemed healthy based on behavior, dietary intake, body condition, and physical examination.

Each rhinoceros received a combination of etorphine (9.8 mg/ml; Novartis, Kempton Park 1619, South Africa), azaperone (40 mg/ml; Janssen Pharmaceutical Ltd., Halfway House 1685, South Africa), and hyaluronidase (5,000 IU/vial; Kyron Laboratories, Benrose 2011, South Africa) delivered into the muscles of the nuchal hump remotely using a 3.0-ml plastic dart with a 60-mm uncollared needle propelled by a compressed air rifle (DAN-INJECT, International S.A., Skukuza 1350, South Africa). Doses were based on standardized age categories: 3 to 4 years = 2.5 mg etorphine, 20 mg azaperone, and 5,000 IU hyaluronidase; 4 to 5 years = 3.0 mg etorphine, 40 mg azaperone, and 5,000 IU hyaluronidase; and ≥5 years = 4 mg etorphine, 40 mg azaperone, and 5,000 IU hyaluronidase. Darting took place early in the morning prior to feeding or cleaning of enclosures to ensure minimal disturbance or stimulation of the animals.

All animals were recumbent within 10 min after darting and were blindfolded as soon as they could be safely approached and placed in sternal recumbency. Each rhinoceros's body position was alternated between sternal and lateral recumbency at 20-min intervals to ensure adequate ventilation of both lung fields and perfusion of limbs, respectively.

At $t = 0$, once the rhinoceros were handled for the first time, an initial blood sample was obtained. Butorphanol (50 mg/ml; Kyron Laboratories) was administered intravenously at 10 times the etorphine dose (mg) into an auricular vein immediately following the collection of samples and assessment of cardiorespiratory parameters at $t = 0$. Arterial blood samples were collected, and heart rate, respiratory rate, and rectal temperature were measured every 10 min starting at $t = 0$, for a total duration of 100 min. Initial handling of recumbent rhinoceros was designated time 0 min ($t = 0$). The first sample collected after the administration of butorphanol was obtained at 10 min ($t = 10$). This single butorphanol dose was selected since it is commonly used in field immobilization of free-ranging white rhinoceros and because subjective results suggest it improves cardiorespiratory functions.[17]

At the end of the procedure, naltrexone (40 mg/ml; Kyron Laboratories) was administered intravenously at 33.3 to 57 times the etorphine dose (mg), and the animal was kept under observation until it had fully recovered.

Sample collection and assays

Arterial samples were collected from the medial auricular artery in a 1-ml heparinized syringe and immediately analyzed using a portable blood gas analyzer (iSTAT®1 Handheld Clinical Analyzer, Heska Corporation, Loveland, Colorado 80538, USA) using the CG4+ cartridge (iSTAT CG4+ cartridges, Heska Corporation). Arterial partial pressure of oxygen (PaO_2), arterial partial pres-

Table 1. Mean, standard deviation, median, interquartile range, and number of observations for each cardiopulmonary parameter at sampling periods 0, 10, and 20 min.*

Sample time (min)	Distribution	Respiratory rate (breaths/min)	Heart rate (beats/min)	Rectal temperature (°C)	BE_{ecf} (mmol/L)	HCO_3^- (mm Hg)
	Mean	7.62	121.46	36.60	9.46	35.25
	SD	2.22	16.52	0.76	3.36	3.61
	Median	7.00	120.00	36.70	8.00	33.50
	IQR	6.00, 8.00	112.00, 130.00	36.15, 37.09	7.00, 12.00	32.70, 37.40
	n	13	13	12	13	13
10	Mean	6.31	86.31	36.71	9.31	35.52
	SD	2.50	21.62	0.84	4.63	4.39
	Median	5.00	96.00	36.75	9.00	35.20
	IQR	5.00, 7.00	68.00, 100.00	36.25, 37.40	5.00, 14.00	31.70, 39.90
	n	13	13	12	13	13
20	Mean	5.92	80.31	36.64	10.15	36.12
	SD	2.72	16.60	0.75	4.88	4.73
	Median	5.00	80.00	36.60	11.00	37.40
	IQR	4.00, 8.00	68.00, 94.00	36.40, 37.20	7.00, 15.00	33.10, 40.30
	n	13	13	13	13	13

* IQR indicates interquartile range (25th–75th percentile); BE_{ecf}, base excess; HCO_3^-, bicarbonate; SaO_2, arterial hemoglobin oxygen saturation; PaO_2, arterial partial pressure of oxygen; $PaCO_2$, arterial partial pressure of carbon dioxide; A-a gradient, alveolar – arterial gradient.

sure of carbon dioxide ($PaCO_2$), pH, and lactate were measured by the machine; PaO_2, $PaCO_2$, and pH were corrected for body temperature. Base excess (BE_{ecf}), bicarbonate (HCO_3^-), and arterial hemoglobin oxygen saturation (SaO_2) were values calculated by the blood gas analyzer. Heart rate was determined by auscultation of the heart or palpation of the medial auricular artery. Respiratory rate was measured by visual assessment of thoracic-abdominal excursions and air movement at the nares. The Alveolar – arterial (A-a) oxygen gradient was calculated using the formula $FIo_2(Pb\text{-}P_{H2O}) - Paco_2 - Pao_2$, as reported by Meyer et al.[16] A measured mean barometric pressure (Pb) of 739 mm Hg and an inspired oxygen fraction (FIo_2) of 21 mm Hg were used in all calculations. The water vapor pressure of saturated air in the alveoli (P_{H2O}) was calculated as $4.58 \exp [(17.27T_b)/(237.3 + T_b)$. Body temperature ($T_b$) was measured by placing a thermometer deep into the rectum against the rectal wall. It was assumed that alveolar partial pressure of carbon dioxide was equilibrated with $PaCO_2$.[16]

Data analysis

STATA (Stata Statistical Software: Release 11, College Station, Texas 77840, USA) was used for the statistical analysis. Means, standard deviations, medians, and first (Q1) and third (Q3) quartile were calculated for descriptive purposes for rhinoceros at different sampling points (10-min intervals). As a result of the relatively small sample size obtained for this study, nonparametric statistical tests were used to compare median values at different sampling points (over 100 min). Initially, the data were screened using the Kruskal-Wallis test to assess if median values for different cardiorespiratory, blood gas, A-a gradient, and lactate values differed over sampling points (over 100 min). Secondly, a pairwise comparison of adjacent intervals (i.e., 0 to 10, 30 to 40, 70 to 80 min, etc.) was conducted to assess differences in median cardiorespiratory, blood gas, A-a gradient, and lactate values within a 10-min period using the Wilcoxon rank sum test. All sampling points were compared to the baseline values at $t = 0$ (before the administration of butorphanol). To account for repeated measurements (lack of independence among samples taken from the same rhinoceros at different sample points and the three rhinoceros sampled twice), a mixed linear regression model using ranks was used to evaluate the effect of time on the cardiorespiratory, blood gas, A-a gradient, and lactate values over time, including the sample intervals (every 10 min), as a fixed effect and using $t = 0$ as the reference value. Based on the initial results and distribution of data (indicating that most clinically relevant changes in cardiorespiratory, blood gas, A-a gradient, and lactate values occurred during the first 20 min and then tended to stabilize), the analysis (mixed linear regression using ranks as described above) was repeated in order to formally assess changes on cardiorespi-

Table 1. Extended.

SaO_2 (%)	Lactate (mmol/L)	Corrected pH	Corrected $PaCO_2$ (mm Hg)	Corrected PaO_2 (mm Hg)	A-a gradient (mm Hg)
51.46	2.88	7.34	65.33	30.83	49.35
18.13	1.72	0.04	11.71	9.36	10.26
47.00	2.48	7.34	60.95	30.00	49.19
44.00, 64.00	1.87, 3.46	7.33, 7.36	58.85, 68.45	25.00, 37.50	39.59, 58.95
13	13	12	12	12	12
74.77	2.10	7.32	69.05	47.31	29.07
17.18	1.87	0.04	10.03	11.00	6.95
79.00	1.50	7.32	64.80	48.00	29.13
76.00, 82.00	1.09, 1.91	7.31, 7.35	60.90, 76.10	42.00, 54.00	26.90, 33.28
13	13	13	13	13	13
73.92	1.13	7.33	68.89	47.00	29.53
14.44	0.71	0.04	9.04	12.63	13.82
74.00	0.89	7.34	68.40	45.00	30.00
66.00, 84.00	0.60, 1.44	7.29, 7.36	67.70, 70.80	39.00, 53.00	23.04, 32.83
13	12	13	13	13	13

ratory, blood gas, A-a gradient, and lactate values after 20 min using t = 20 as the reference value. To summarize the data for the last 80 min of the immobilization procedure (between 20 and 100 min), the bootstrap method was used to obtain the median (and median 95% confidence interval [CI]) for each parameter. Box plot graphs were used to present the distribution of data for selected parameters over the 100-min period. Statistical significance was set at $P < 0.05$ for all statistical tests.

RESULTS

Median cardiorespiratory values for rhinoceros at initial sampling (t = 0) were as follows: respiratory rate = 7 breaths per min (breaths/min), PaO_2 = 30 mm Hg, SaO_2 = 47%, $PaCO_2$ = 60.95 mm Hg, A-a gradient = 49.19, mm Hg and heart rate = 120 beats/min. Median arterial blood pH was 7.34, BE_{ecf} = 8 mM/L, HCO_3^- = 33.5 mM/L, lactate = 2.48 mM/L, and rectal temperature = 36.7°C (Table 1). Rhinoceros also initially exhibited muscle tremors and limb rigidity. The mean induction time from darting to recumbency for all rhinoceros in the study was 5.71 min (95% CI, 4.69–6.72).

At t = 10 and t = 20 there were a number of statistically significant changes in median values of physiologic parameters when compared to t = 0 (Table 1). Subjective observations also showed improved muscle relaxation, with a reduction in tremors. PaO_2 (48 and 45 mm Hg, respectively) and SaO_2 (79% and 74%, respectively) both increased significantly at t = 10 and t = 20 min ($P < 0.0001$), and A-a gradient (29.13 and 30.00 mm Hg, respectively) decreased significantly at the same time points ($P < 0.001$). Heart rate decreased significantly to 96 beats/min at 10 min and 80 beats/min at 20 min, compared to 120 beats/min at t = 0 ($P < 0.001$). Compared to values at t = 0 (2.48 mM/L), lactate levels were lower at t = 10 (1.50 mM/L, $P = 0.019$) and t = 20 (0.89 mM/L, $P < 0.001$). Respiratory rate decreased from 7 breaths/min at t = 0 to 5 breaths/min at t = 10 ($P = 0.005$) and remained at 5 breaths/min at t = 20 ($P < 0.0001$). At t = 10, a significant difference was observed in median pH (7.32) when compared to the value at t = 0 (7.34). There were no significant changes at t = 10 and t = 20 in median rectal temperatures, BE_{ecf}, HCO_3^-, and $PaCO_2$ compared to the corresponding values at t = 0.

During the latter periods of immobilization (20–100 min), median values for respiratory rate and $PaCO_2$ (Fig. 1) did not change significantly, although both SaO_2 and PaO_2 remained at higher levels compared to the values at t = 0 (Fig. 2). Compared to t = 20 (74%), SaO_2 was significantly ($P < 0.05$) different at t = 50, 60, 70, 90, and 100, with median oxygen saturation levels at 88%, 89%, 84%, 91%, and 85%, respectively. PaO_2 values followed the same trend as SaO_2. PaO_2 was significantly ($P < 0.05$) different at t = 50, 60, 90, and 100, with median values at 57, 61, 59, and 52 mm Hg, respectively, compared to values at t = 20 (45 mm Hg). A-a gradients at t = 50, 60, and 90 min were significantly ($P < 0.05$) different from t = 20 (30 mm Hg), with values of 20.37, 20.98, and 20.07 mm Hg, respectively. There was

Table 2. Summary distribution (mean, standard deviation, minimum, 25th percentile [Q1], median, 75th percentile [Q3], maximum, 95% confidence interval [CI] for the median, and number of observations) for each cardiopulmonary parameter over the last 80 min (between 20 and 100 min) of the immobilization.[a]

Estimate	Respiratory rate (breaths/min)	Heart rate (beats/min)	Rectal temperature (°C)	BE_{ecf} (mmol/L)	HCO_3^- (mm Hg)
Mean	5.60	71.40	36.50	13.60	38.77
Standard deviation	2.45	15.53	0.77	4.62	4.28
Minimum	2.00	36.00	35.00	1.00	25.90
Q1	4.00	60.00	36.00	9.00	35.10
Median	5.00	71.00	36.40	14.00	39.60
Q3	6.00	80.00	36.90	17.00	42.20
Maximum	16.00	114.00	38.30	22.00	47.40
Median 95% CI[b]	3.7–6.3	64.1–79.9	36.1–36.7	9.3–18.7	35.8–43.4
n	94	94	94	93	93

[a] BE_{ecf}, base excess; HCO_3^-, bicarbonate; SaO_2, arterial hemoglobin oxygen saturation; PaO_2, arterial partial pressure of oxygen; $PaCO_2$, arterial partial pressure of carbon dioxide; A-a gradient, alveolar – arterial gradient.

[b] The median 95% CIs were obtained using the bootstrap method to account for lack of independence due to repeated measurement in the same rhinoceros over time.

an overall decreasing trend in heart rate, with significant differences observed at t = 50 and t = 100 (72 and 62 beats/min, respectively; $P < 0.003$). Median rectal temperature decreased from 36.7°C (t = 20) to 36.4°C (t = 100), although this change was not statistically significant ($P = 0.07$).

Median pH values improved from 7.34 (t = 20) to 7.37 (t = 90) ($P = 0.001$). Other significant changes in acid–base status between 20 and 100 min included increases in BE_{ecf} and HCO_3^- and a decrease in lactate. BE_{ecf} continued to rise after t = 20 (11 mM/L), with significant differences observed at t = 40 and beyond (17 and 16 mM/L at t = 80 and t = 100, respectively; $P \leq 0.015$). Bicarbonate ions also increased over time, with median values at t = 50 and later (41.35 mM/L at t = 100) significantly ($P \leq 0.011$) higher than values at t = 20 (37.4 mM/L). Lactate values after 20 min continued decreasing, and median values were significantly different at t = 80 (0.68 mM/L; $P = 0.033$), t = 90 (0.46 mM/L; $P = 0.023$), and t = 100 (0.80 mM/L; $P = 0.006$), compared to the value at t = 20 (0.89 mM/L) (Fig. 3). A summary distribution for each cardiopulmonary parameter over the last 80 min of immobilization is shown in Table 2.

DISCUSSION

The purpose of this study was to evaluate the cardiorespiratory, blood gas, and lactate values in white rhinoceros immobilized for a prolonged period of time with a single dose of butorphanol administered intravenously and in the absence of supplementary oxygen. Historically, white rhinoceros have only been kept immobilized for short time periods (≤20 min) as a result of the perceived

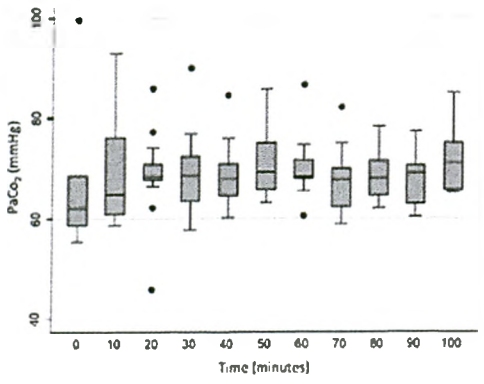


Figure 1. Distribution of $PaCO_2$ values over a 100-min immobilization of white rhinoceros.

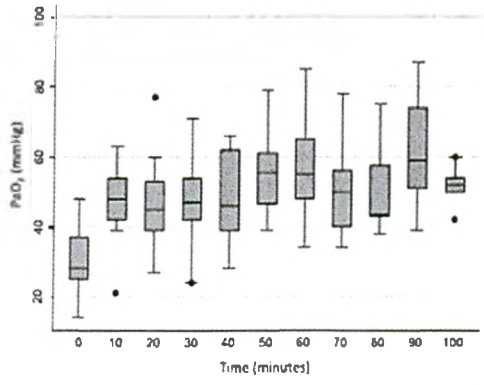


Figure 2. Distribution of PaO_2 values over a 100-min immobilization of white rhinoceros.

Table 2. Extended.

SaO_2 (%)	Lactate (mmol/L)	Corrected pH	Corrected PaO_2 (mm Hg)	Corrected $PaCO_2$ (mm Hg)	A-a gradient (mm Hg)
80.02	1.06	7.35	51.90	69.20	24.70
12.40	0.78	0.04	13.30	7.10	10.40
35.00	0.30	7.25	24.00	45.80	0.16
74.00	0.50	7.33	42.50	65.20	17.30
83.00	0.90	7.36	50.50	68.50	25.60
89.00	1.30	7.38	60.00	72.20	32.80
96.00	4.70	7.41	87.00	89.80	64.10
76.9–89.1	0.6–1.2	7.3–7.4	43.8–58.1	65.3–71.7	21.2–30.0
93	92	92	92	92	91

threats associated with severe opioid-induced respiratory depression.[1,22]

The initial observations of hypopnea, hypoxemia, hypercapnia, and tachycardia are consistent with other reports of immobilized rhinoceros.[2,4,17,28] Hypoxemia occurs when $PaO_2 \leq 80$ mm Hg; levels reaching 50–60 mm Hg usually require corrective action in an anesthetized animal.[31] The elevated $PaCO_2$ (median $PaCO_2 = 60.95$; normal range = 44.4–53.7 mm Hg[4]) at the first sampling period indicated that the hypoxemia (median $PaO_2 = 30$ mm Hg; normal range = 90.2–108.6 mm Hg[4]) was, in part, caused by hypoventilation.[11,29] Since the PCO_2 values of alveolar gas and arterial blood in healthy individuals are almost identical, changes in $PaCO_2$ are indicative of variations in alveolar ventilation, with hypoventilation resulting in increased arterial carbon dioxide values.[29] As alveolar ventilation decreases and $PaCO_2$ increases, there will be a corresponding drop in PaO_2 levels as rates of alveolar oxygen replenishment are reduced. Hypoventilation is a pronounced side effect associated with the administration of opioids and is mediated by the activation of MOP, KOP, and DOP receptors, reducing the sensitivity of carotid and aortic bodies to hypoxemia and, more significantly, the sensitivity of brainstem chemosensory neurons to increasing carbon dioxide levels.[15,18] In addition, opioids have depressant effects on respiratory neurons in the brainstem, causing alterations to rhythmogenesis, with resulting hypopnea.[31] Opioids can further compromise ventilation through increased chest wall rigidity, which limits changes in intrathoracic pressure, and the expansion and return to rest of lungs in the immobilized animal.[22] Reduced upper airway patency following the administration of opioids can further compromise alveolar ventilation because of increased resistance to air movements through the respiratory tree.[28,31]

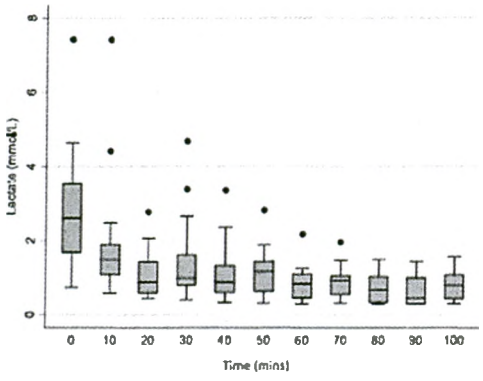


Figure 3. Distribution of lactate values over a 100-min immobilization of white rhinoceros.

The high A-a gradient (median A-a gradient = 49.19 mm Hg; normal equine A-a gradient = approximately 10 mm Hg[7]) suggests that ventilation/perfusion mismatching (V/Q ratio), a physiologic right-to-left shunt, and diffusion impairment may have also contributed to the low oxygen tension.[11,29] A lower V/Q ratio (proportion of air to blood reaching an alveoli) for a particular lung area will impair pulmonary gas exchange and result in a lower PaO_2. As a result of the "S" shape of the oxygen–hemoglobin dissociation curve, areas of high V/Q ratios have limited effect on arterial PaO_2.[29] A shunt (V/Q ratio = 0) occurs when blood flows past unventilated alveoli or in pulmonary tissue not associated with alveoli, resulting in no gaseous exchange. This unoxygenated blood flows from the arterial to the venous pulmonary circulation, leading to a lower PaO_2.[15,29] Opioids decrease PaO_2 through reduced

alveolar ventilation and by decreasing pulmonary perfusion. Pulmonary vasoconstriction occurs with opioids because of both hypoxia and direct effects on vasculature.[16] A decreased V/Q ratio, including shunts, will also impair carbon dioxide excretion; normally this does not result in an increased arterial carbon dioxide tension, since as $PaCO_2$ rises, respiration is stimulated. However, in the immobilized animal, because of the opioid-induced respiratory inhibition, an increase in $PaCO_2$ would be expected. The impact of immobilizing drugs used in rhinoceros on the hypoxic pulmonary vasomotor response is unknown, although injectable anaesthetics, including narcotics, barbiturates, and benzodiazepines, examined in domestic animals do not have any detectable effect.[15,29] Large anesthetized recumbent animals are subject to ventilation–perfusion disparities due to lung compression by body mass and alterations to tidal volume from the abdominal organs impinging on the diaphragm.[19,21] Diffusion of oxygen may be impaired by an increase in the physical separation between alveolar gas and pulmonary capillary blood (caused by interstitial or pulmonary edema) and a shortened pulmonary transit time of blood. Since the rhinoceros in this study were clinically healthy, it is believed they did not have any lung pathology that may have reduced diffusion. However, it has been demonstrated in goats that etorphine causes an increase in the A-a gradient due to pulmonary hypertension, and it is proposed that this may result in pulmonary edema, which impairs the diffusion of oxygen (Meyer, pers. comm.). It may also reduce the time for gaseous exchange because of a reduced blood transit time through the alveolar capillaries.

Arterial oxygen levels (PaO_2 and SaO_2) improved markedly in the first 10 min following the administration of butorphanol in the immobilized white rhinoceros. Significant differences in these variables did not occur again until later in the immobilization period ($t = 50$ and beyond) and were not as large as the initial change. These findings suggest that the initial improvement in arterial oxygen tension was due to the administration of butorphanol rather than to an effect of time reducing the immobilizing drug effects through metabolism and redistribution, as likely occurred later in the immobilization (Tables 1, 2). Improved alveolar ventilation would increase PaO_2 levels; however, there was no evidence to indicate an increase in ventilation, since $PaCO_2$ values did not change significantly during the extended immobilization.[29] Increased PaO_2 without concurrent changes in $PaCO_2$ indicates that etorphine-induced respiratory depression was not antagonized by the administration of butorphanol. The significant reduction in the A-a gradient from $t = 0$ to $t = 10$ may also have contributed to the increase in PaO_2 through potential improvements in the V/Q ratio, shunt fraction, diffusion of oxygen, or various combinations of all three.

Although the results of these clinical observations do not elucidate the mechanism responsible for the improved PaO_2, a number of possibilities are proposed. Butorphanol is reported to reduce muscle rigidity and trembling of limbs in rhinoceros associated with the administration of etorphine, which would reduce tissue oxygen requirements.[23] Decreased tissue oxygen demands, without changes in pulmonary gaseous exchange, can lead to increased blood oxygen levels.[15] Azaperone, included in the initial immobilizing drug combination, may also have caused muscle relaxation, as maximum activity of this drug is reached within 20 to 30 min after administration.[1] However, it has been observed by the author (PB) during capture of large numbers of rhinoceros ($\geq$500) with etorphine and azaperone that muscle tremors and rigidity persist through the immobilization period if butorphanol is not administered. Recent studies in boma-confined white rhinoceros immobilized with etorphine and azaperone without butorphanol administration, in which muscle tremors persisted, did not show an increase in PaO_2 over 20 min.[11] As a partial mixed opioid agonist–antagonist, butorphanol may also relieve muscle rigidity, which impairs the movement of the thoracic wall and diminishes upper airway patency.[2,21,28] It could also counteract etorphine-associated pulmonary vasoconstriction, resulting in improved pulmonary gas exchange and, hence, blood oxygenation.[16]

Despite persistently high $PaCO_2$ levels, respiratory rate decreased significantly in the first 10 min and then remained at this level for the remaining 90 min. This may have been due to ongoing drug-induced respiratory depression by the initial immobilizing drugs or the KOP receptor agonist effects of butorphanol.[24] However, the decrease in respiratory rate was associated with improved blood oxygen values. Possible explanations for this change in PaO_2 include decreased tissue metabolism and oxygen consumption, an increase in tidal volume with a fractional decrease in alveolar dead space ventilation, an improvement in the A-a gradient, or various combinations of all three.

In the exercising animal, increased lactate values are used as a measure of anaerobic metabolism due to hypoxia or tissue hypoperfusion. The highest median lactate value (2.48 mM/L) occurred at t = 0 but was lower than that observed in field-immobilized white rhinoceros.[17] This is not unexpected, as free-ranging animals are usually darted from a helicopter and experience high levels of muscle activity prior to and during the immobilization induction phase compared to the boma-confined study animals.[19] Normal resting lactate values in white rhinoceros are not available in the literature; however, values for horses (0.70–2.85 mM/L) suggest the lactate levels in these rhinoceros were resting or slightly elevated.[21] An initial increase in lactate production may have occurred as a result of opioid-induced localized muscle activity and limb rigidity (tremors) combined with hypoxemia during the induction phase.[1] A decrease in anaerobic metabolism and muscle activity in the immobilized animal would result in progressively decreasing lactate levels.

Despite a persistent hypercapnia, lactate levels and other acid–base parameters improved over time. The initial moderate acidosis improved as a result of increased BE_{ecf} and HCO_3^- and decreased lactic acid (Fig. 3; Tables 1, 2). These results indicate that the improvement in acid–base status was metabolic in origin rather than dependent on respiratory compensatory mechanisms.

The rhinoceros in this study were tachycardic at t = 0 despite minimal exertion associated with darting in a confined holding space. Etorphine is reported to cause both tachycardia and bradycardia depending on dose, species, and other concurrently administered drugs.[3,12] Pronounced tachycardia and increased blood pressure have been reported in elephants after administration of etorphine.[7,10] At t = 20, the median heart rate in rhinoceros was reduced by approximately one-third from the initial tachycardia (median 120 beats/min). This reduction may be due to a partially reversed hypoxemic vasodilator effect associated with increases in PaO_2 and SaO_2 or a decline in the sympathetic response to hypoxia.[8,9] Another possibility is a direct opioid receptor-mediated effect on the heart.[6] A more complete understanding of the observed heart rate changes would require measures of cardiac output, stroke volume, and total peripheral resistance. The α_1-adrenoceptor antagonist activity of azaperone on peripheral vasculature should also be taken into account.

There is a paucity of literature regarding the effects of butorphanol on the cardiovascular system. In humans, butorphanol reduces tachycardia in patients under the influence of cocaine; the explanation for this observation has not been elucidated.[30]

The underlying mechanism for changes in PaO_2, SaO_2, and respiratory and heart rates during the first 20 min requires further investigation. Although the timing of observed changes in this report indicates that butorphanol may have contributed to these effects, interpretation is limited by the lack of a control group (i.e., animals to which no butorphanol was administered), which was not included because of the requirements of a concurrent study using these individual animals. Anecdotal observations suggest butorphanol improves immobilization quality and physiologic parameters in white rhinoceros.[22] However, the specific behavioral, pharmacologic, and therapeutic effects of this mixed opioid agonist–antagonist are not clearly defined in complex biological systems. The clinical effects of butorphanol in immobilized rhinoceros are further complicated by the presence of etorphine, a potent pure agonist at all three opioid peptide receptors.[31] In humans, the actions of butorphanol are similar to those of pentazocine, which does not antagonize the respiratory depression produced by morphine. In postoperative patients, 2 to 3 mg of butorphanol produced a respiratory depression equivalent to that of 10 mg of morphine.[31] Butorphanol administered to rhesus monkeys resulted in a dose-dependent decrease in respiratory minute volume and behavioral effects consistent with MOP receptor activity, which overrides KOP receptor-mediated actions.[27,30] The receptor binding profile of butorphanol also varies between species, and the precise mechanism of action is not fully elucidated. Pharmacologic effects in rhesus monkeys and pigeons are produced through MOP receptors, compared to both MOP and KOP receptors in laboratory rodents.[30] It has previously been reported[28] that butorphanol did not result in any benefits to ventilation in immobilized white rhinoceros. However, the effects of butorphanol may have been dampened in this study by the addition of detomidine to the etorphine and azaperone combination. Detomidine, an α_2-agonist, can cause significant negative respiratory effects, compounding those of the opioids.[28]

As a result of the severe hypoxemia that occurs in opioid-immobilized white rhinoceros, it has been suggested that oxygen supplementation

should be routinely used; however, this can be difficult to accomplish. Under extensive conditions, animals are frequently immobilized many kilometers from a home base with limited personnel and equipment associated with helicopter capture. Orotracheal intubation can be demanding in white rhinoceros because of the size of the head and the muscle rigidity associated with the use of etorphine, which makes it difficult to open the mouth.[2] Intermittent positive pressure ventilation requires the placement of a cuffed endotracheal tube and use of high-capacity ventilators or a demand valve connected to a large source of compressed air.[2] Oxygen supplementation into the trachea using an equine nasogastric tube passed through the nasal cavity has also been described.[2]

A recent study[11] has shown that the oxygen supplementation in white rhinoceros immobilized with etorphine and azaperone does not improve the resulting hypoxemia and causes further increases in $PaCO_2$ in animals that are already hypercapnic and a severe acidemia with worsening blood pH. It is hypothesized that these effects were due to increased intrapulmonary shunt fractions resulting in lung atelectasis. It is also possible that the high levels of hypercarbia, which result from increasing $PaCO_2$ levels in animals receiving oxygen, may depress central nervous system (CNS) respiratory control.[11] However, if butorphanol was administered prior to oxygen supplementation, hypoxemia was completely reversed, although neither $PaCO_2$ nor pH improved.[11] Similar results have been reported[2] in rhinoceros administered nalorphine (mixed opioid agonist–antagonist) or doxapram hydrochloride (CNS stimulant) prior to or during the intratracheal administration of supplementary oxygen to the level of the carina. Oxygen saturation increased to greater than 90%, although hypercapnia and academia persisted. In humans, supplemental oxygen exacerbates opioid-induced ventilatory depression, which may be due to decreased output of the peripheral and ventral medulla chemoreceptors, causing a reduced ventilation drive.[20]

CONCLUSION

Despite the relatively small sample size in this report ($n = 13$ white rhinoceros immobilizations), several statistically significant and clinically relevant changes occurred during the immobilization period. Initial severe hypoxemia in rhinoceros immobilized with etorphine and azaperone and administered a single i.v. dose of butorphanol (at 10 times the etorphine dose) at $t = 0$ improved during the first 20 min, as reflected by increased PaO_2 and SaO_2. However, the change in blood oxygen tension was limited, and animals remained hypoxic. Steadily declining lactate values indicate a lack of generalized anaerobic metabolism, which suggests that tissue oxygen delivery met metabolic demand or that metabolic rate and oxygen consumption was reduced during the first 20 min, or both.[14] $PaCO_2$ values did not change significantly, and hypercapnia persisted for the entire 100 min; however, during this time, acidosis was corrected by metabolic compensatory mechanisms. A well-designed study with controls is required to further investigate these initial results, which suggest that butorphanol may improve blood oxygen tension and provide some cardiovascular and acid-base support in immobilized rhinoceros. Factors such as body position and other potential confounders (e.g., body mass, age, drug dosages, and activity levels prior to drug administration) should also be considered. Observations indicate that healthy white rhinoceros immobilized with etorphine and azaperone and administered a single dose of butorphanol can tolerate prolonged periods (100 min) of hypoxemia and hypercapnia.

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CHAPTER 3

Post-induction butorphanol administration alters oxygen consumption to improve blood gases in etorphine-immobilized white rhinoceros

Buss, P., Miller, M., Fuller, A., Haw, A., Stout, E., Olea-Popelka, F. & Meyer, L.

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Post-induction butorphanol administration alters oxygen consumption to improve blood gases in etorphine-immobilized white rhinoceros

Peter Buss[*,‡,#], B.V.Sc., M.Med.Vet., Michele Miller[†], D.V.M., Ph.D., Andrea Fuller[‡,#] B.Sc. (Hons), Ph.D., Anna Haw[‡], B.V.Sc., Ph.D., Eliza Stout[§], B.Sc., Francisco Olea-Popelka[§], D.V.M., Ph.D., and Leith Meyer[‡,#], B.V.Sc., Ph.D.

[*]Veterinary Wildlife Services, South African National Parks, Kruger National Park, Private Bag X402, Skukuza 1350, South Africa; [†]Department of Science and Technology/National Research Foundation Centre of Excellence for Biomedical Tuberculosis Research, Medical Research Council Centre for TB Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town 8000, South Africa. [‡]Brain Function Research Group, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, South Africa; [§]Colorado State University, College of Veterinary Medicine and Biomedical Science, Department of Clinical Sciences, Fort Collins, Colorado 80523, USA; [#]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0002, South Africa; Correspondence should be addressed to Dr. Peter Buss (peter.buss@sanparks.org, +27 82 905 4665).

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Abstract

Objective To investigate the effects of post-induction butorphanol administration in etorphine-immobilized white rhinoceros on respiration and blood gases.

Study design Randomized crossover study.

Animals Six sub-adult male white rhinoceros.

Methods Etorphine, or etorphine followed by butorphanol 12 minutes after recumbency, was administered intramuscularly [2.5 mg etorphine, 25 mg butorphanol (1000 to 1250 kg), or 3.0 mg etorphine, 30 mg butorphanol (1250 to 1500 kg)]. Sampling started at 10 minutes after initial recumbency, and was repeated at five-minute intervals for 25 minutes. Arterial blood gases, limb muscle tremors, expired minute ventilation and respiratory frequency were measured at each sampling point. Calculated values included alveolar to arterial oxygen gradient, expected respiratory minute volume, tidal volume, physiological deadspace, oxygen consumption, and carbon dioxide production.

Results Etorphine administration resulted in an initial hypoxaemia median (range) [PaO_2 25.0 (23-28) mmHg], hypercapnia [$PaCO_2$ 76.2 (67.2-81.2) mmHg], increased P(A-a) O_2 [41.7 (36.6-45.1) mmHg, $\dot{V}O_2$ [11.1 (10.0-12.0) L $minute^{-1}$] and muscle tremors. Butorphanol administration was followed by rapid, although moderate, improvements in PaO_2 [48.5 (42-51) mmHg] and $PaCO_2$ [62.8 (57.9-75.2) mmHg]. In rhinoceros receiving butorphanol, median $\dot{V}O_2$ [4.4 (3.6-5.1) L $minute^{-1}$] and [$\dot{V}CO_2$ (4.2 (3.8-4.4) L $minute^{-1}$] were lower than in those not receiving butorphanol. Increased arterial oxygen tension was associated with lower oxygen consumption (p=0.002) which was positively associated with lower muscle tremor scores (p<0.0001).

Conclusion and clinical relevance Hypoxaemia and hypercapnia in etorphine-immobilized rhinoceros resulted from an increased alveolar-arterial gradient and increased oxygen consumption and carbon dioxide production associated with muscle tremors. Rather than being associated with changes in respiratory minute ventilation, it appears that improved blood gases following butorphanol administration were a consequence of decreased oxygen consumption associated with reduced muscle tremoring.

Keywords butorphanol, etorphine, oxygen consumption, white rhinoceros

Introduction

Chemical capture is an essential tool in the management of free-ranging white rhinoceros (*Ceratotherium simum*) (Wenger et al. 2007). Etorphine, the opioid preferentially used in immobilization of rhinoceros, results in areflexia without total loss of consciousness, due to central nervous system depression within a few minutes following intramuscular (IM) administration (Portas 2004; Swan 1993). Etorphine is commonly combined with azaperone, a butyrophenone tranquillizer, which reduces induction times and opioid-associated hypertension (Portas 2004). Unfortunately, hypoxaemia, hypercapnia and acidaemia are well documented adverse effects associated with the use of etorphine and azaperone, and mortalities have been associated with immobilization in rhinoceros (Kock et al. 1995; Haw et al. 2015). Butorphanol, a mixed opioid agonist - antagonist, is administered intravenously (IV) in immobilized rhinoceros to mitigate these adverse cardiorespiratory effects. However, inconsistent changes in blood gases following butorphanol administration have been reported. Different explanations for the effect of butorphanol in white rhinoceros, including improved ventilation or possible alterations in metabolic activity, also have been proposed (Miller et al. 2013; Haw et al. 2014; Boardman et al. 2014; Buss et al. 2015). The objectives of this study were to determine the effects of etorphine on respiration in immobilized rhinoceros and changes that occur following the intravenous administration of butorphanol. Cardiovascular effects of etorphine and intravenous butorphanol in these study rhinoceros have been previously reported (Buss et al. 2016).

Materials and methods

The study had ethical approval from SANParks Animal Use and Care Committee (Ref. no. 14-2) and University of the Witwatersrand Animal Ethics Screening Committee (Ref. no. 2014/15/C). Management of the rhinoceros was conducted according to the South African National Parks (SANParks) Standard Operating

Procedures for the Capture, Transportation and Maintenance in Holding Facilities of Wildlife.

Six white rhinoceros, sub-adult (5 to 6 year old) males, were captured in Kruger National Park (23° 49' 60 S, 31° 30' 0 E; alt. 317m), South Africa, and habituated to captivity in holding pens over a period of four months. The study was a crossover design with two interventions allocated using computer generated random numbers with a two week washout period between treatments; I) etorphine hydrochloride (9.8 mg mL^{-1}, M99, Elanco, Gauteng, South Africa) plus hyaluronidase (5000 i.u., Kyron Laboratories, Gauteng, South Africa), administered intramuscularly (IM), and II) etorphine hydrochloride plus hyaluronidase administered IM followed by butorphanol (50 mg mL^{-1}, Kyron Laboratories) administered IV. Doses were 2.5 mg etorphine, 5000 i.u. hyaluronidase, 25 mg butorphanol (1000 to 1250 kg), and 3.0 mg etorphine, 5000 i.u. hyaluronidase, 30 mg butorphanol (1250 to 1500 kg) (Haw et al. 2014; Buss et al. 2015). Azaperone was not included in the immobilizing drug combination due to potential confounding respiratory and cardiovascular effects (Portas 2004).

Etorphine plus hyaluronidase were administered using a 3.0 mL plastic dart with a 60 mm un-collared needle propelled from a compressed air rifle (DAN-INJECT, International S.A., Skukuza 1350, South Africa). Once an animal could be safely handled, it was blindfolded and placed in sternal recumbency for one minute to facilitate initial instrumentation and subsequently rolled into lateral recumbency. The influence of variable induction times on physiological measurements was reduced by conducting a trial only if the rhinoceros became recumbent and could be safely handled within 15 minutes after darting (Haw et al. 2014). Recumbency was used as an indicator of immobilization level equivalency between trials. Data collection started 10 minutes after initial recumbency (t = 0), and was repeated at five-minute intervals over a 25 minutes study period. In those rhinoceros receiving treatment II, butorphanol was administered IV at 2 minutes (t = 2) as this allowed for instrumentation of study animals and most closely approximated the time at which butorphanol is administered in field-immobilized rhinoceros. In treatment I, rhinoceros were administered sterile saline at t=2. At

the end of each trial, all rhinoceros were weighed and administered naltrexone (40 mg mL^{-1}, Kyron laboratories) IV at 20 times the etorphine dose.

Expired minute ventilation (corrected for body temperature and saturated pressure) ($\dot{V}E_{BTPS}$) (L $minute^{-1}$) was measured via shortened equine endotracheal (ET) tubes (V KRUUSE I.D. 28, CAT. No. 282270, Jørgen Kruuse A/S, Langeskov, Denmark) inserted into each nostril with the cuffs inflated to create an airtight seal. A two-way Y-shape non-rebreathing valve (2730 Series, Hans Rudolph, inc, Shawnee, USA) was connected to each ET tube at the nares external margin which allowed inspired air to enter the tube and directed expired air to a PowerLab Exercise Physiology System (ML870B80, ADInstruments, Castle Hill, Australia). Expired minute ventilation ($\dot{V}E_{BTPS}$) was determined via a respiratory flow head (MLT1000L) linked to a spirometer (ML140) and a gas mixing chamber (MLA245). Expired air temperature was recorded by a thermistor pod (ML309) in the mixing chamber.

Expired air was collected into a Douglas bag for one minute at the end of each sampling interval and analysed using a Cardiocap/5 (Datex-Ohmeda, GE Healthcare, Helsinki, Finland) for mixed-expired carbon dioxide pressure ($P\bar{E}CO_2$) (mmHg) and expired oxygen fraction (FEO_2) (%). The same monitor was used to analyse expired air from one of the ET tubes to determine end-tidal carbon dioxide pressure ($PETCO_2$) (mmHg) and oxygen fraction ($FETO_2$) (%), and measure respiratory frequency (f_R). Body temperature (TB) (°C) was measured using a rectal thermometer (BAT-12, Physitemp Instruments, New Jersey, USA).

A 22 gauge intravenous catheter was inserted into an auricular artery and blood samples collected into heparinized 1mL syringes and immediately analysed using a portable blood gas analyser (iSTAT 1 Handheld Clinical Analyzer, Heska Corporation, Colorado, USA) and CG4+ cartridge (iSTAT CG4+ cartridges, Heska Corporation). The alveolar – arterial oxygen gradient ($P(A\text{-}a)O_2$) (mmHg) was calculated using the formula $FIO_2(PB - PH_2O) - PaCO_2 - PaO_2$, with inspired oxygen fraction (FIO_2) (%) standardized to 20.9 % and barometric pressure (PB) (mmHg) measured by the portable blood gas analyser prior to each immobilization. Alveolar vapour pressure of saturated air (PH_2O) (mmHg), at a

specific TB, was determined using the formula 4.58 exp [(17.27 TB)/(237.3 + TB)] (Meyer et al. 2010). The expected respiratory minute ventilation ($\dot{V}EXP$) (L minute^{-1}) in the rhinoceros prior to immobilization was estimated from body mass using the formula 0.518 $BM^{0.802}$ (Bide et al. 1997). Actual respiratory minute ventilation was considered to be equivalent to $\dot{V}E_{BTPS}$, which was divided by f_R to calculate tidal volume (VT) (L breath^{-1}).

The Enghoff modified Bohr's equation $((PaCO_2 - P\bar{E}CO_2)/PaCO_2)VT$ was used to determine physiological dead space ($\dot{V}D_{PHYS}$) (L breath^{-1}) (Tusman et al. 2012). The ($\dot{V}D_{PHYS}$) was corrected by 300 mL for the volume of the two ET tubes extending beyond the rhinoceros nostrils.

Oxygen consumption ($\dot{V}O_2$) (L minute^{-1}) was calculated as the difference between inspired and expired oxygen fractions as a proportion of expired minute ventilation at standard temperature and dry pressure ($\dot{V}E_{STPD}$), i.e. $\dot{V}O_2 = (FIO_2 - FEO_2)/100$ x ($\dot{V}E_{STPD}$) (McArdle et al. 1986). The $\dot{V}E_{BTPS}$ was multiplied by (273/310)((PB -47)/760) to convert from BTPS to STPD (West 2008). Since inspired and expired minute ventilation were not equivalent (depending on the respiratory quotient), and $\dot{V}E_{STPD}$ was used to determine both FIO_2 and FEO_2, the Haldane transformation was used to correct the inspired oxygen volume, i.e. $\dot{V}O_2 = \dot{V}E_{STPD}(FIO_2((1-(FEO_2 + FECO_2)/1 - (FIO_2 + FICO_2)) - FEO_2)$ (McArdle et al. 1986).

Carbon dioxide production ($\dot{V}CO_2$) (L minute^{-1}) was calculated as the product of $\dot{V}E_{STPD}$ and the difference between expired and inspired carbon dioxide fractions. Carbon dioxide production was determined using the formula $\dot{V}CO_2 = \dot{V}E_{STPD}(FECO_2 - 0.03\%)$. Inspired fractions for oxygen and carbon dioxide were standardized at $FIO_2 = 20.9\%$ and $FICO_2 = 0.03\%$ (McArdle et al. 1986).

Skeletal muscle tremors, especially of the limbs, head, and shoulders, in the immobilized rhinoceros were subjectively evaluated and scored by a single observer at each time point according to criteria in Annexure I. Total muscle tremor scores were calculated as the sum of all the scores for that treatment at each time point.

Data analyses

STATA (Stata Statistical Software: Release 14, College Station, Texas, USA) was used for statistical analyses. Descriptive statistics were calculated to assess data distribution for each treatment at different sampling points. Due to the relatively small sample size (n=6), non-parametric statistical tests were used to compare median blood gases and respiratory values at specific sampling points within each treatment. The Kruskal-Wallis test was used to assess whether median values for blood gases and respiratory parameters differed over sampling points. Based on these findings, differences in medians between matched pairs of values at t=0 to t=10 were compared using the Wilcoxon rank signed test. To confirm that no further changes occurred after 10 minutes, linear regression (using ranks) was used to assess changes in blood gases and respiratory parameters after 10 minutes using t=10 as the reference value. Correlations between blood gases, respiratory parameters and muscle tremor scores were evaluated using linear regression. To evaluate differences in blood gases and respiratory parameters between treatment groups, linear regression (using ranks) was used to compare median blood gases and respiratory parameters while adjusting for the effect of time. Statistical significance was set at $p < 0.05$ for all statistical tests.

Results

All rhinoceros in both treatments became sternally recumbent within 15 minutes of etorphine administration, and sample and data collection started 10 minutes later. All study animals recovered with no ill effects.

Treatment I: etorphine

Table 1 shows the blood gases and respiratory values for treatment I. Median arterial PaO_2 and $PaCO_2$ did not change significantly during the first 10 minutes (t=0 to t=10) or subsequent 15 minutes (t=10 to t =25) (Fig. 1).

The median $\dot{V}EXP$ (t=0) was 163 L minutes^{-1}. $\dot{V}E_{BTPS}$ declined between t= 0-10 and t=10-25; however, these changes was not statistically significant (Fig. 2). The f_R decreased significantly (p=0.034) over the first ten minutes, but did not

change between t=10-25. No changes over time (t= 0-10, t=10-25) were observed in VT, P(A-a) O_2 and $\dot{V}D_{PHYS}$.

The $\dot{V}O_2$ decreased between t=0 and t=10 (p=0.046) (Figure 3A); however the decrease in $\dot{V}CO_2$ over the same period was not statistically significant (Figure 3B). There was a positive correlation between $PaCO_2$ and $\dot{V}CO_2$ (p=0.021, r^2 =0.15), and inverse correlations between PaO_2 and $\dot{V}O_2$ (p=0.038, r^2 =0.12). Both $\dot{V}O_2$ and $\dot{V}CO_2$ were associated with muscle tremor scores (p<0.0001, r^2 = 0.52; p=0.0001, r^2=0.57, respectively) which decreased over the study period (Fig. 4).

Treatment II: etorphine and butorphanol

Table 1 shows the blood gases and respiratory values for treatment II. In etorphine-immobilized rhinoceros administered butorphanol IV (t=2), there was a significant increase in PaO_2 (p=0.027) and decrease in PaCO2 (p=0.046) between t=0 and t=10 with no further significant changes (Fig. 1). Between t=0 and t=10, $\dot{V}E_{BTPS}$ declined (p=0.03) with a decrease in f_R (p=0.032), a reduction in V_T (p=0.046) and drop in $\dot{V}D_{PHYS}$ (p=0.03), then remained unchanged (t=10-25) (Fig. 2). Following butorphanol administration, there was a transient increase in $\dot{V}E_{BTPS}$ which reached a maximum value at t=3, but returned to pre-injection values at t=5. The P(A-a) O_2 did not change significantly during the trial. The $\dot{V}O_2$ declined between t=0 and t=10 (p=0.027) with no further changes over time (Fig. 3A). Trends in $\dot{V}CO_2$ were similar to $\dot{V}O_2$ with declines between t=0 and t=10 (p=0.027), although there was also a decrease from t=10 to t=25 (p=0.014) (Figure 3B). There was a positive association between $PaCO_2$ and $\dot{V}CO_2$ (p=0.0002; r^2=0.34), and inverse correlations between PaO_2 and $\dot{V}O_2$ (p=0.002, r^2 =0.25). Both $\dot{V}O_2$ and $\dot{V}CO_2$ were associated with muscle tremor scores (p<0.0001, r^2 =0.72; p<0.0001, r^2=0.65, respectively). Muscle tremors decreased rapidly between t=0 and t=5 and remained low for the rest of the trial period (Fig. 4).

Table 1. Distribution of blood gases and respiratory parameters, median and interquartile range (25th to 75th percentile), at sampling periods 0, 5, 10, and 25 minutes in six captive male white rhinoceros (5 to 6 year old) for two treatments: I) etorphine IM; and, II) etorphine IM and butorphanol IV.

	Treatment[a]	0 min		5 min		10 min		25 min	
PaO_2 (mmHg)	I	25.0	(23-28)	25.0	(23-28)	27.5	(23-29)	26.0	(25-29)
(kPa)		3.3	(3.1-3.7)	3.3	(3.1-3.7)	3.7	(3.1-3.9)	3.5	(3.3-3.9)
	II	25.5	(22-26)	48.0	(42-50)	48.5	(42-51)	43.5	(38-46)
		3.4	(2.9-3.5)	6.4	(5.6-6.7)	6.5	(5.6-6.8)	5.8	(5.1-6.1)
$PaCO_2$ (mmHg)	I	76.2	(67.2-81.2)	71.1	(60.9-79.6)	72.3	(66.9-81.8)	78.5	(70.8-82.4)
(kPa)		10.2	(9.0-10.8)	9.5	(8.1-10.6)	9.6	(8.9-10.9)	10.5	(9.4-11.0)
	II	81.7	(76.1-89.3)	58.2	(54.7-68.4)	62.8	(57.9-75.2)	63.7	(62.8-65)
		10.9	(10.1-11.9)	7.8	(7.8-9.1)	8.4	(7.7-10.0)	8.5	(8.4-8.7)
$\dot{V}E_{BTPS}$ (L minute^{-1})	I	164.0	(126.6-182.2)	137.6	(102.5-153.7)	118.7	(88.5-130.6)	96.1	(66.8-101.4)
	II	151.4	(139.0-172.2)	153.0	(125.7-160.5)	89.5	(85.0-98.5)	83.0	(76.6-86.5)
fR (breaths minute^{-1})	I	9.5	(9-10)	8.5	(8-9)	7.0	(6-7)	5.5	(5-8)
	II	9.5	(8-10)	11.0	(10-13)	7.5	(6-8)	6.0	(6-7)
VT (L breath^{-1})	I	18.0	(14.1-21.5)	16.1	(14.0-16.7)	18.3	(17.7-18.7)	14.2	(12.7-15.5)
	II	16.8	(15.2-20.4)	12.2	(11.7-16.1)	11.9	(11.0-16.9)	13.4	(11.7-14.4)
P(A-a) O_2 (mmHg)	I	41.7	(36.6-45.1)	48.9	(39.0-53.3)	44.3	(33.8-46.9)	39.0	(33.6-44.1)
(kPa)		5.6	(4.9-6.1)	6.5	(5.2-7.1)	5.9	(4.5-6.3)	5.2	(4.5-5.9)
	II	37.0	(33.3-41.5)	38.9	(32.9-43.1)	37.0	(30.7-39.3)	36.7	(35.0-39.1)
		4.9	(4.4-5.5)	5.2	(4.4-5.7)	4.9	(4.1-5.2)	4.9	(4.7-5.2)
$\dot{V}D_{PHYS}$ (L minute^{-1})	I	59.5	(35.8-69.8)	42.1	(19.3-60.5)	39.5	(23.5-43.7)	35.6	(26.4-48.2)
	II	47.9	(46.9-50.9)	35.9	(17.5-52.2)	31.4	(21.9-41.2)	29.0	(26.1-34.5)
$\dot{V}O_2$ (L minute^{-1})	I	11.1	(10.0-12.0)	8.7	(7.7-9.8)	7.8	(5.8-8.6)	6.4	(3.7-7)
	II	10.9	(9.1-12.0)	6.8	(5.5-8.0)	4.4	(3.6-5.1)	4.2	(4.0-4.6)
$\dot{V}CO_2$ (L minute^{-1})	I	8.3	(7.8-11.4)	6.8	(5.9-7.8)	6.0	(4.9-7.1)	4.3	(3.5-5.3)
	II	9.3	(7.2-10.4)	6.9	(6.2-7.7)	4.2	(3.8-4.4)	3.7	(2.7-3.8)

[a]Treatment I, etorphine IM (2.5 mg etorphine, 1000 to 1250 kg; 3.0 mg etorphine, 1250 to 1500 kg); Treatment II, etorphine IM (2.5 mg etorphine 1000 to 1250 kg; 3.0 mg etorphine 1250 to 1500 kg) and butorphanol IV (25 mg butorphanol, 1000 to 1250 kg; 30 mg butorphanol, 1250 to 1500 kg).

Intramuscular (IM), intravenous (IV), arterial partial pressure oxygen (PaO_2) and carbon dioxide ($PaCO_2$), expired minute ventilation ($\dot{V}E_{BTPS}$), respiratory rate (fR), tidal volume (VT), alveolar –arterial oxygen gradient (P(A-a) O_2, physiological dead space ($\dot{V}D_{PHYS}$), oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$).

Comparison of treatments I and II

Table 2 shows overall distribution of blood gases and respiratory values between treatments I and II. The PaO_2 was higher ($p<0.001$) and $PaCO_2$ was lower ($p=0.001$) when comparing overall median values in treatment II *versus* treatment I (Fig. 1). There were no differences in median values for $\dot{V}E_{BTPS}$ or $\dot{V}D_{PHYS}$ between treatments (Fig. 2). Median f_R was statistically higher ($p=0.045$) and V_T significantly lower ($p=0.008$) in animals receiving butorphanol. In treatment II, median P(A-a) O_2 values were lower compared to treatment I ($p=0.019$). Overall $\dot{V}O_2$ and $\dot{V}CO_2$ were lower in treatment II compared to treatment I animals ($p=0.001$) (Figs. 3A & 3B).

Table 2. Overall distribution of blood gases and respiratory parameters, median and interquartile range (25th to 75th percentile), over the sampling period 0 to 25 minutes in six captive male white rhinoceros (5 to 6 year old) for two treatments: I) etorphine IM; and, II) etorphine IM and butorphanol IV.

[a]Treatment	I		II	
PaO_2 (mmHg)	26	(23-29)	42.5	(30.5-48.5)
(kPa)	3.5	(3.1-3.9)	5.7	(4.1-6.5)
$PaCO_2$ (mmHg)	75.5	(67.4-82.3)	64.3	(61.1-76.1)
(kPa)	10.1	(9.0-11.0)	8.6	(8.1-10.1)
$VE_{(BTPS)}$ (L minute^{-1})	105.9	(84.3-137.9)	90.0	(84.7-137.9)
f_R (breaths minute^{-1})	7.0	(5-7)	7.0	(6-9)
V_T (L minute^{-1})	16.3	(14.0-18.3)	14.1	(11.8-16.2)
P(A-a) O_2 (mmHg)	41.5	(34.1-45.8)	36.8	(33.2-40.3)
(kPa)	5.5	(4.5-6.1)	4.9	(4.4-5.4)
VO_2 (L minute^{-1})	7.3	(5.6-8.7)	4.8	(4.1-6.8)
VCO_2 (L minute^{-1})	5.8	(4.2-7.2)	4.1	(3.8-6.3)

[a]Treatment I, etorphine IM (2.5 mg etorphine, 1000 to 1250 kg; 3.0 mg etorphine, 1250 to 1500 kg); Treatment II, etorphine IM (2.5 mg etorphine 1000 to 1250 kg; 3.0 mg etorphine 1250 to 1500 kg) and butorphanol IV (25 mg butorphanol, 1000 to 1250 kg; 30 mg butorphanol, 1250 to 1500 kg).

Intramuscular (IM), intravenous (IV), arterial partial pressure oxygen (PaO_2) and carbon dioxide ($PaCO_2$), expired minute ventilation ($\dot{V}E_{BTPS}$), respiratory rate (fR), tidal volume (VT), alveolar –arterial oxygen gradient (P(A-a) O_2, physiological dead space ($\dot{V}D_{PHYS}$), oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$).

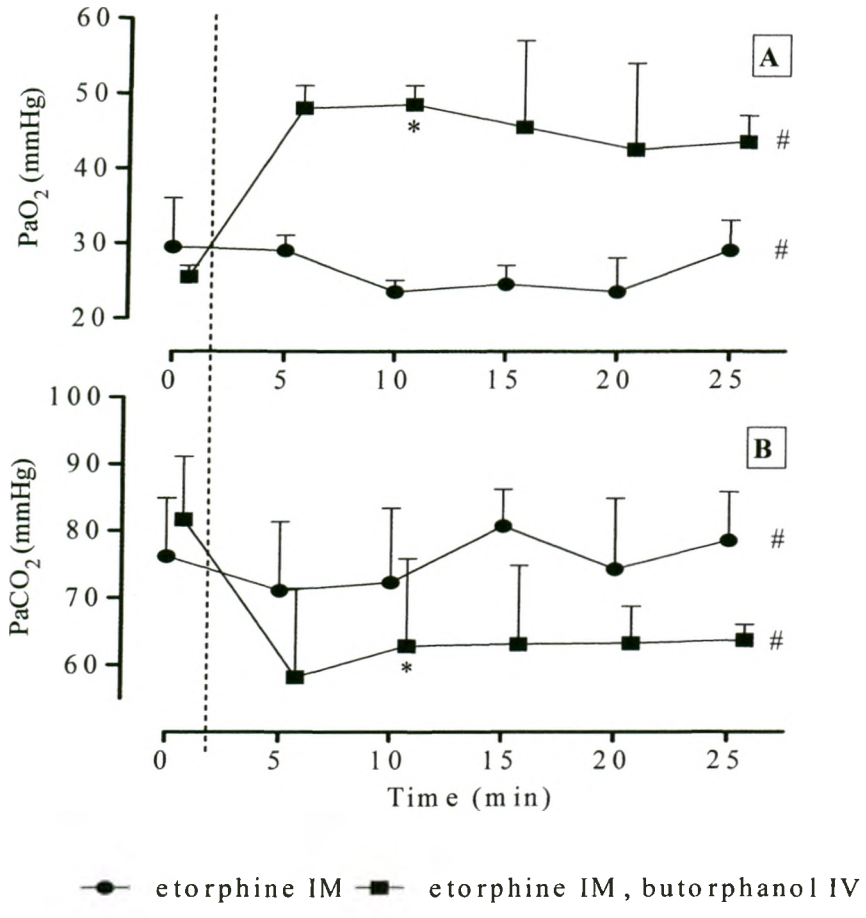


Figure 1. Median and interquartile range of arterial partial pressures of (A) oxygen (PaO_2) and (B) carbon dioxide ($PaCO_2$) at sampling periods 0, 5, 10, 15, 20 and 25 minutes in six captive male white rhinoceros (5 to 6 years old) for treatment I (etorphine IM) or treatment II (etorphine IM and butorphanol IV). The dashed line indicates the time at which butorphanol was administered.

*, indicates a significant ($p < 0.05$) difference within treatment between $t = 0$ and $t = 10$,

+, indicates a significant ($p < 0.05$) difference within treatment between $t = 10$ and $t = 25$,

#, indicates a significant ($p < 0.05$) difference in overall median values between treatments I and II from 5 to 25 minutes.

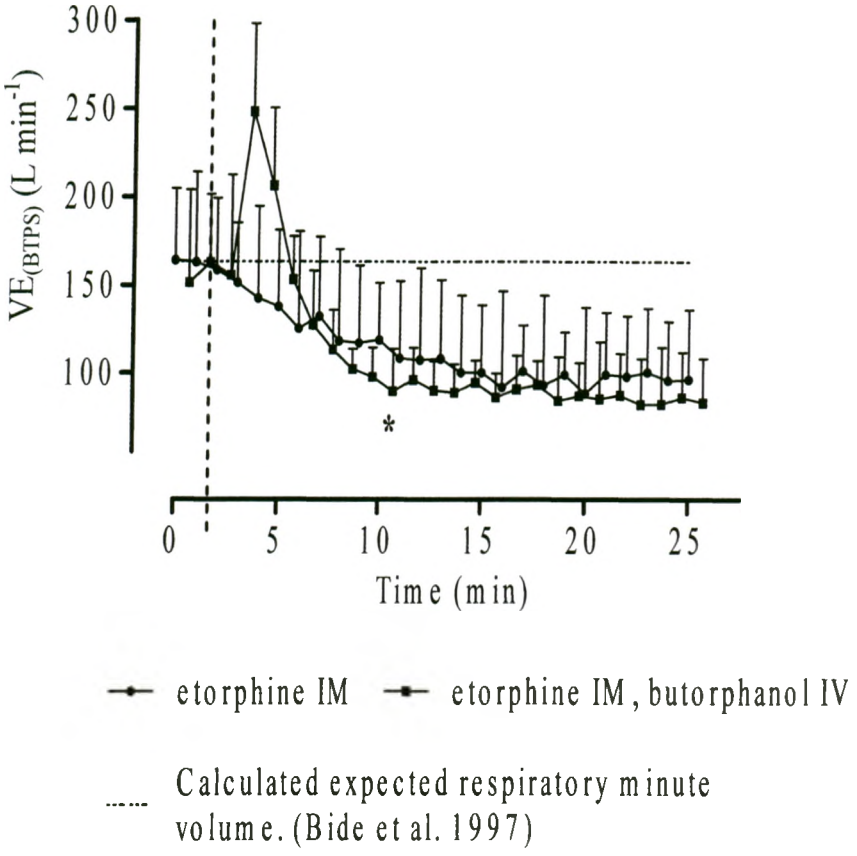


Figure 2. Median and interquartile range of expired minute ventilation ($\dot{V}E_{BTPS}$) measured at one minute intervals between 0 and 25 minutes in six captive male white rhinoceros (5 to 6 years old) for treatment I (etorphine IM) or treatment II (etorphine IM and butorphanol IV). The dashed line indicates the time at which butorphanol was administered.

*, indicates a significant ($p < 0.05$) difference within treatment between $t = 0$ and $t = 10$,

+, indicates a significant ($p < 0.05$) difference within treatment between $t = 10$ and $t = 25$,

#, indicates a significant ($p < 0.05$) difference in overall median values between treatments I and II from 5 to 25 minutes.

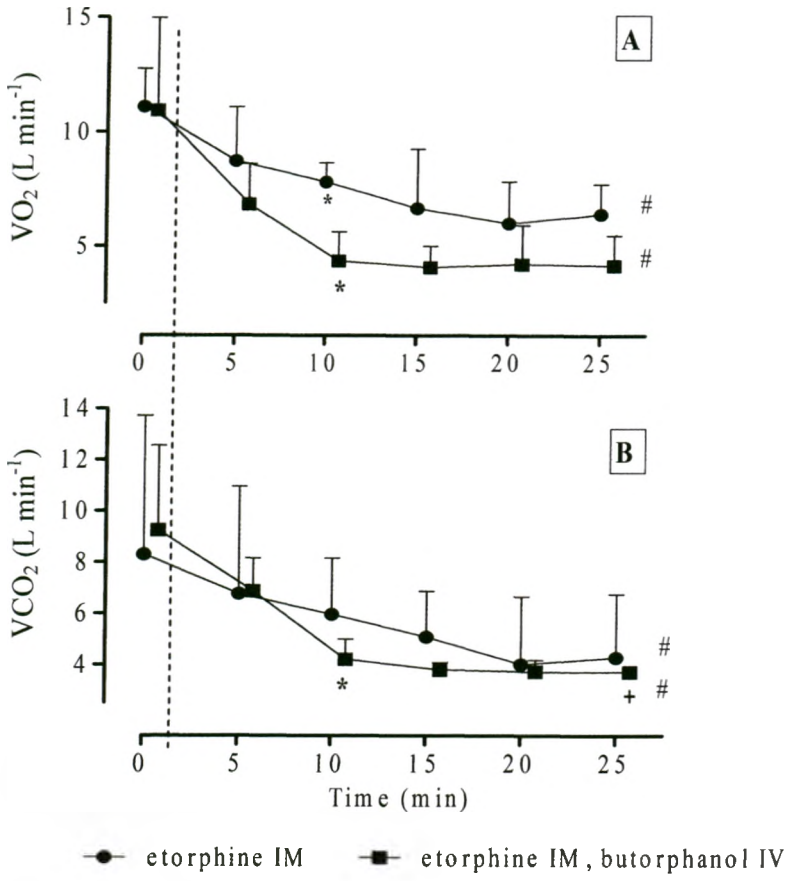


Figure 3. Median and interquartile range of (A) oxygen consumption ($\dot{V}O_2$) and (B) carbon dioxide production ($\dot{V}CO_2$) calculated for sampling periods 0, 5, 10, 15, 20 and 25 minutes in six captive male white rhinoceros (5 to 6 years old) for treatment I (etorphine IM) or treatment II (etorphine IM and butorphanol IV). The dashed line indicates the time at which butorphanol was administered.

*, indicates a significant ($p < 0.05$) difference within treatment between t = 0 and t = 10,

+, indicates a significant ($p < 0.05$) difference within treatment between t =10 and t = 25,

#, indicates a significant ($p < 0.05$) difference in overall median values between treatments I and II from 5 to 25 minutes.

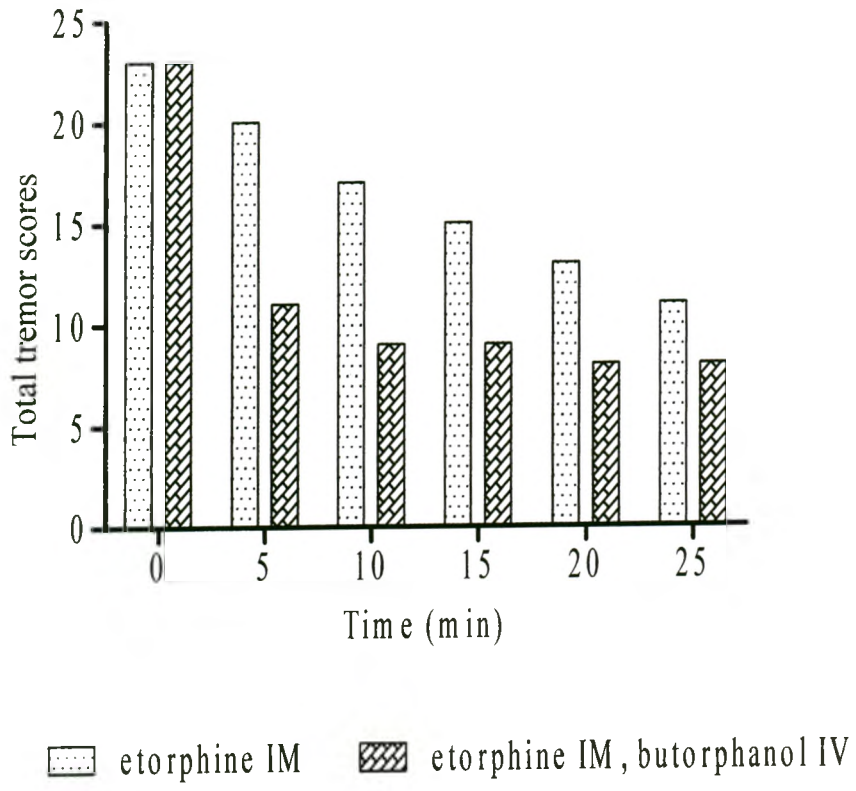


Figure 4. Muscle tremor scores at sampling periods 0, 5, 10, 15, 20 and 25 minutes were the sum of all the scores (1 to 5) at each time point in six captive male white rhinoceros (5 to 6 years old) for treatment I (etorphine IM) or treatment II (etorphine IM and butorphanol IV).

Discussion

In this study, immobilization of white rhinoceros with etorphine resulted in hypoxaemia and hypercapnia. Contrary to previous reports, these changes were not associated with a decrease in respiratory minute ventilation but rather an increase in alveolar-arterial oxygen gradient and oxygen consumption. Administration of IV butorphanol was followed by improvements in arterial oxygen and carbon dioxide tensions, although animals remained hypoxaemic and hypercapnic. Improved blood gases appeared to result primarily from a decrease in oxygen consumption associated with decreased muscle tremors, rather than from changes in ventilation.

The use of only six rhinoceros due to welfare considerations and logistical challenges is a study limitation. The small sample size may have resulted in lack of statistical differences and masked potentially clinically important physiological changes. Inability to determine alveolar ventilation, cardiac output, pulmonary artery pressures, shunt fractions and $\dot{V}/\dot{Q}$ ratios, in part, limited a comprehensive understanding of physiological mechanisms influencing arterial blood gases. Physiological differences may exist between free-ranging and captive rhinoceros; therefore further studies should compare these conditions.

Hypoxaemia and hypercapnia are frequently reported in etorphine-immobilized rhinoceros and extremes of blood gases measured in our study (PaO_2 of about 25 mmHg and $PaCO_2$ of about 80 mmHg) suggest they can be life-threatening (Yaksh & Wallace 2011; Haw et al. 2014; Boardman et al. 2014). A $PaO_2 \leq 80$ mmHg indicates hypoxaemia in an anaesthetized animal and animals with values below 60 mm Hg normally require supportive treatment (Read 2003). A $PaCO_2 > 70$ mmHg may produce myocardial depression, arrhythmias and impaired metabolism due to respiratory acidosis, and ventilator support is advocated (Moens 2013). In addition, opioid chemoreceptor depression can profoundly depress hypercapnic and hypoxic ventilatory responses (Pattinson 2008).

In etorphine-immobilized rhinoceros, initial median $\dot{V}E_{BTPS}$ was clinically similar to $\dot{V}EXP$ at rest. These results suggest that the marked hypoxaemia and hypercapnia measured in the first arterial sample were not a consequence of a

reduction in ventilation, as has been previously advocated (Kock et al. 1995; Miller et al. 2013; Boardman et al. 2014; Haw et al. 2014). It is unlikely that the absence of reduced $\dot{V}E_{BTPS}$ was due to a delay in maximum respiratory opioid effect as etorphine had caused sufficient central nervous system depression to induce a state of immobilization and recumbency for 10 minutes prior to sampling. Meyer et al. (2015) reported similar results in that hypoventilation was not the primary cause of hypoxaemia and hypercapnia in etorphine-immobilized domestic goats.

The initial median f_R (9.5 breaths minutes^{-1}) was substantially lower than the rate (16 to 23 breaths minutes^{-1}) reported for standing unrestrained captive white rhinoceros (Citino et al. 2007). These values suggest that $\dot{V}E_{BTPS}$ was maintained by an increase in VT in the immobilized rhinoceros. In the study rhinoceros, the increased VT may have been a compensatory response to hypoxaemia and, or, hypercapnia. It is also possible that initial sympathetic stimulation associated with darting influenced respiratory minute ventilation (Heistad et al, 1972).

In rhinoceros immobilized only with etorphine, hypoxaemia and hypercapnia did not change significantly over the 25 minute study interval. Although median $\dot{V}E_{BTPS}$ also did not change significantly, the initial and final values of 164 and 96 L minute^{-1}, respectively, may reflect a clinically relevant reduction in minute volume. A decrease in $\dot{V}E_{BTPS}$ is contradictory to the expected response to hypercapnia and hypoxia (Pattinson, 2008). Opioids suppress the ventilatory response to hypercapnia and hypoxia through reduced excitation of chemosensory neurons and chemoreceptor bodies (Yaksh & Wallace 2011). It is possible that etorphine receptor binding increased over time, causing further depression of respiratory rhythmogenesis resulting in hypopnoea; however, it is likely that peak respiratory perturbations had been reached at initial sample collection in the immobilized rhinoceros at approximately 20 minutes post-dart (Yaksh & Wallace 2011). The decrease in $\dot{V}E_{BTPS}$ may have been due to an opioid-induced diminished hypoxic drive associated with a lowered set-point. An arterial oxygen tension of 25 mmHg in immobilized rhinoceros should have stimulated an increase in ventilation, which was not observed. In opioid-

immobilized rhinoceros, the hypothetically lowered hypoxic threshold would have resulted in a decreased respiratory stimulus at PaO_2 > 25 mmHg with a subsequent fall in ventilation and limited changes in PaO_2 (Pattinson 2008).

The elevated P(A-a) O_2 observed at t=0 may have contributed to decreased PaO_2 and increased $PaCO_2$. The normal resting A-a gradient is unknown for white rhinoceros; however, a gradient of 41.7 mmHg in the immobilized rhinoceros, compared to 10 mmHg in horses at rest, suggests a clinically relevant finding (Doherty & Valverdeis 2008). An elevated A-a gradient is indicative of ventilation/perfusion mismatching, a physiologic right-to-left shunt, or impaired diffusion of gases between alveoli and perfusing blood (West 2008). A decreased $\dot{V}/\dot{Q}$ ratio does not usually result in hypercapnia since increasing $PaCO_2$ stimulates respiration. However, this response may be limited in opioid-immobilized rhinoceros due to alterations in chemoreceptor sensitivity (Buss et al. 2015). Etorphine-induced pulmonary hypertension may contribute to hypoxaemia by hindering gas exchange across alveolar-capillary membranes due to pulmonary congestion with interstitial oedema or a decrease in blood flow passage time through pulmonary vasculature (Meyer et al. 2015).

Oxygen consumption is a measure of metabolic activity and maintenance of homeostatic processes in mammals (Porter 1995). The $\dot{V}O_2$ was elevated in etorphine-immobilized rhinoceros (8.23 ml kg^{-1} $minute^{-1}$) compared to horses (3 ml kg^{-1} $minute^{-1}$) at rest and may have contributed significantly to hypoxaemia (Evans & Rose 1988). The difference in $\dot{V}O_2$ may be due to species differences; however, metabolic rate per unit body mass tends to decrease with increased size, so a rhinoceros should consume less oxygen per unit body mass than should a horse (Porter 1995). Increased skeletal muscle activity may be an important contributor to metabolic demands, an idea supported by our findings of a strong correlation between $\dot{V}O_2$ and muscle tremor scores in immobilized rhinoceros.

The overall decrease in $\dot{V}E_{BTPS}$ over time in treatment II supports the hypothesis that IV administration of butorphanol in etorphine-immobilized rhinoceros does not improve respiratory minute ventilation. The $\dot{V}E_{BTPS}$ increased for a few minutes following the administration of butorphanol then subsequently

decreased, similar to treatment I, so overall there was no significant difference between the two treatments. The $\dot{V}D_{PHYS}$ was also similar between treatments, suggesting changes in deadspace ventilation did not influence blood gases following butorphanol administration. The P(A-a) O_2 was generally lower in treatment II compared to treatment I, but an initial lower value and no significant change following butorphanol administration, suggests this finding is of limited clinical importance. Further investigations are needed to confirm the consistency and significance of this difference between treatments as changes in alveolar ventilation: perfusion ratios, shunting, and gas diffusion rates across alveolar-capillary membranes could alter arterial blood gases (Haw et al. 2014; West, 2008).

The results of this study suggest that the improvements in PaO_2 and $PaCO_2$, following butorphanol administration in etorphine-immobilized white rhinoceros, arose from changes in metabolic oxygen consumption associated with decreased muscle tremors. An inverse correlation between PaO_2 and $\dot{V}O_2$, and a positive correlation between $\dot{V}O_2$ and muscle tremor scores, suggest that these three variables are interrelated. We propose that an increase in PaO_2 following IV butorphanol administration is a result of decreased metabolism associated with reduced muscle tremors. Carbon dioxide production followed a similar trend to that of oxygen consumption which further supports the hypothesis that blood gas changes following butorphanol administration result from reduced metabolic activity. In shivering humans recovering from induced hypothermia, it has been shown that carbon dioxide production and oxygen consumption are positively associated with muscular activity and metabolic rate (Ralley et al. 1988). A reduction in muscle tremors associated with butorphanol administration may be mediated through antagonism of etorphine-induced sympathetic nervous system activity. Muscle tremors in immobilized rhinoceros are significantly associated with increased plasma catecholamine concentration (de Lange 2015). An alternative explanation for the improved arterial blood gases following butorphanol administration may be increased pulmonary perfusion and reduction in $\dot{V}/\dot{Q}$ inequality rather than changes in oxygen consumption (Wagner 2008). However, heart rate and systemic blood pressure decreased in etorphine-

immobilized rhinoceros following butorphanol administration, suggesting a decrease in cardiac output (Buss et al. 2016).

The transient increase in ventilation following the administration of butorphanol is of clinical significance in that it has the potential to mislead the uninformed observer. It may appear that butorphanol administration improves ventilation by increasing respiratory rate; however, within a few minutes, respiratory minute ventilation was the same as in opioid-immobilized rhinoceros that did not receive butorphanol.

Conclusion

Our findings support the hypothesis that butorphanol does not improve respiratory minute ventilation in etorphine-immobilized white rhinoceros. However, butorphanol did improve arterial oxygen and carbon dioxide tensions, likely as a consequence of reduced metabolism and muscle tremors. We also showed that hypoxaemia and hypercapnia following etorphine administration were not a result of decreased respiratory minute ventilation. An increase in the alveolar-arterial oxygen gradient likely contributed to hypoxia in immobilized white rhinoceros. However, the impact of and underlying physiological processes leading to changes in A-a gradient require further elucidation. Our findings provide evidence that hypoxia and hypercapnia in immobilized rhinoceros result also from increases in metabolic oxygen consumption and carbon dioxide production with inadequate ventilatory compensation.

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Annexure I: Muscle tremor scores

Criteria for subjectively scoring muscle tremors.

Degree of Muscle Tremor	
Level **5**:	Severe Tremors – resulting in whole body and head movement
Level **4**:	Moderate Tremors – resulting in severe shoulder, chest, leg and foot movement
Level **3**:	Slight Tremors – resulting in minor shoulder, chest and severe leg and foot movement
Level **2**:	Mild Tremors – resulting in minor leg and foot movement
Level **1**:	No Visible Tremors

CHAPTER 4

Cardiovascular effects of etorphine, azaperone, and butorphanol combinations in chemically immobilized captive white rhinoceros (*Ceratotherium simum*).

Buss, P., Miller, M., Fuller, A., Haw, A., Wanty, R., Olea-Popelka, F. & Meyer, L.

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CARDIOVASCULAR EFFECTS OF ETORPHINE, AZAPERONE, AND BUTORPHANOL COMBINATIONS IN CHEMICALLY IMMOBILIZED CAPTIVE WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*)

Peter Buss, B.V.Sc., M.Med.Vet., Michele Miller, D.V.M., Ph.D., Andrea Fuller, B.Sc. (Hons.), Ph.D., Anna Haw, B.V.Sc., Rachel Wanty, B.Sc., Francisco Olea-Popelka, D.V.M., Ph.D., and Leith Meyer, B.V.Sc., Ph.D.

Abstract: Chemical capture is an essential tool in the management and conservation of white rhinoceros (*Ceratotherium simum*); however, cardiovascular responses in immobilized megaherbivores are poorly understood. Blood pressure and heart rate responses in rhinoceros immobilized with etorphine or etorphine plus azaperone, and the effects of subsequent i.v. butorphanol administration were investigated. Six white rhinoceros were used in a randomized crossover study design with four interventions: 1) etorphine i.m.; 2) etorphine plus azaperone i.m.; 3) etorphine i.m. and butorphanol i.v.; and 4) etorphine plus azaperone i.m., and butorphanol i.v. Etorphine resulted in hypertension and tachycardia in immobilized rhinoceros on initial measurements. Over the 25-min study period, blood pressures and heart rate declined. Heart rates were slower, although the rhinoceros were still tachycardic, and blood pressures lower during the whole study period in animals immobilized with etorphine and azaperone compared with those that received only etorphine. Butorphanol administration resulted in lower arterial blood pressures and heart rates in etorphine-immobilized rhinoceros. In rhinoceros immobilized with etorphine and azaperone, heart rate slowed following administration of butorphanol i.v., although blood pressures remained unchanged. Azaperone reduced hypertension associated with etorphine immobilization, but animals remained tachycardic. Administration of butorphanol to etorphine/azaperone-immoblized rhinoceros lowered heart rate to values approaching normal resting levels without altering blood pressure.

Key words: Blood pressure, cardiovascular, *Ceratotherium simum*, heart rate, white rhinoceros.

INTRODUCTION

Chemical immobilization is an essential management tool for moving white rhinoceros (*Ceratotherium simum*) between isolated populations to maintain genetic diversity, collecting biological samples, attaching radio-tracking devices, facilitating dehorning procedures, and treating injured individuals.[22,32] Etorphine plus azaperone, the preferred immobilizing drug combination, causes significant respiratory depression in immobilized white rhinoceros and butorphanol is commonly administered to counteract these effects.[2–4,22,23] Alterations in respiratory function have been extensively studied in white rhinoceros; however, there are only a few reports on the cardiovascular effects of these drugs.[14,22,25,32] The pressor effects of etorphine, and etorphine plus fentanyl, have been described in captive and free-ranging rhinoceros, respectively.[13,15,18] Alterations to heart rate and blood pressure, measured noninvasively, also have been described briefly in game-ranched rhinoceros chemically captured utilizing etorphine, azaperone, and butorphanol.[2] Clinical observations following the administration of butorphanol in immobilized free-ranging rhinoceros suggest that it results in a substantial reduction in heart rates (Buss, pers. comm.).

The purpose of this study was to determine and compare the cardiovascular effects of etorphine or etorphine plus azaperone in boma-adapted

From the Veterinary Wildlife Services, South African National Parks, Kruger National Park, Private Bag X402, Skukuza 1350, South Africa (Buss); Department of Science and Technology/National Research Foundation Centre of Excellence for Biomedical Tuberculosis Research, Medicine Research Council of South Africa Centre for Tuberculosis Research, Faculty of Medicine and Health Sciences, Stellenbosch University, P.O. Box 241, Cape Town 8000, South Africa (Miller); Brain Function Research Group, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Medical School, 7 York Road, Parktown 2193, South Africa (Fuller, Haw); Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Science, Colorado State University, Fort Collins, Colorado 80523, USA (Olea-Popelka, Wanty); Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa (Meyer). Correspondence should be addressed to Michele Miller (michelemiller128@gmail.com).

white rhinoceros, and to evaluate how i.v. butorphanol administration influences these effects.

MATERIALS AND METHODS

Six subadult (5–6 yr) male white rhinoceros, weighing between 1,194 and 1,420 kg, were captured in Kruger National Park (23°49′60 S, 31°30′0 E; alt. 317 m), South Africa, and habituated to captivity over a period of 4 mo. The animals were housed individually in rhinoceros-specific holding pens. Water and a 50 : 50 mix of Lucerne (*Medicago sativa*) and Tef (*Eragrostis tef*) hay were provided ad libitum. Feces were removed from the enclosures, water troughs cleaned, and food replaced daily.

A randomized crossover study design was used with a 2-wk washout period between each of four treatments: treatment 1: etorphine (Elanco, Kempton Park 1619, South Africa; 9.8 mg/ml) plus hyaluronidase (Kyron Laboratories, Benrose 2094, South Africa; 5000 i.u./vial) i.m.; treatment 2: etorphine and azaperone (Janssen Pharmaceutical Ltd., Halfway House 1685, South Africa; 40 mg/ml) plus hyaluronidase i.m.; treatment 3: etorphine plus hyaluronidase i.m. followed by butorphanol (Kyron Laboratories; 50 mg/ml) i.v.; and treatment 4: etorphine and azaperone plus hyaluronidase i.m. followed by butorphanol i.v. Doses were based on two standardized body mass categories: 1,000 to 1,250 kg received 2.5 mg etorphine, 37.5 mg azaperone, 5,000 i.u. hyaluronidase, and 25 mg butorphanol; and 1,250 to 1,500 kg received 3.0 mg etorphine, 45 mg azaperone, 5,000 i.u. hyaluronidase, and 30 mg butorphanol. Hyaluronidase (5,000 i.u.) was included in the immobilizing drug mixture as it facilitates drug absorption and reduces induction time.[10]

The immobilizing drugs were delivered into the muscles of the nuchal hump using a 3.0-ml plastic dart with a 60-mm uncollared needle using a compressed air rifle (DAN-INJECT International S.A., Skukuza 1350, South Africa). Once a darted animal could be safely approached, it was blindfolded and placed into lateral recumbency. Trials were conducted only if the animal was recumbent, could be safely handled within 15 min of darting, and instrumented within a further 10 min. A 22 G × 1-inch catheter (Nipro Safelet Cath, Nipro Medical Corporation, Bridgewater, New Jersey 08807, USA) was placed into a medial auricular artery. Arterial blood pressures were recorded using a transducer (TranStar 60-inch Single Monitoring Kit, Ref MX950T, Smiths Medical ASD, Inc., Dublin, Ohio 43017, USA) secured at the level of the heart and zeroed prior to connecting to a precalibrated Cardiocap/5 physiological monitor (Datex-Ohmeda, GE Healthcare, Helsinki 00510, Finland). Heart rate was determined by chest auscultation and confirmed using the physiological monitor.

Systolic, diastolic, and mean arterial blood pressures, and heart rate were initially recorded at 10 min after the animal became recumbent (t = 0) and at each subsequent 5-min interval for a total of 25 min. Butorphanol (at 10 × the etorphine dose in mg) was administered i.v. into an auricular vein at 2 min (t = 2) after the first measurement (t = 0) to rhinoceros in treatments 3 and 4. An equivalent volume of sterile saline was administered i.v. as a control at t = 2 to those animals not receiving butorphanol.

At the end of each procedure, butorphanol was administered i.v. (at 10 × the etorphine dose mg) to animals in treatments 1 and 2 after the last sample at t = 25 min. All animals were walked into a crate of known weight and weighed by suspending the crate from a scale. Naltrexone (Kyron laboratories; 40 mg/ml) was administered i.v. (at 20 × the etorphine dose in mg). Rhinoceros were kept under observation until fully recovered.

Data analysis

STATA (Stata Statistical Software: Release 14, College Station, Texas 77840, USA) was used for the statistical analysis. Descriptive statistics (means, standard deviations, medians, and 1st [Q1] and 3rd [Q3] quartile) were calculated to assess the data distribution for each treatment at different sampling points. Due to the relatively small sample size used for this study (n = 6), nonparametric statistical tests were used to compare median cardiovascular values at different sampling points within each treatment. Initially, the data was screened using the Kruskal-Wallis test to assess if median values for different cardiovascular parameters differed over sampling points. Subsequently, we formally tested the hypothesis that cardiovascular values changed at t = 10 as compared with t = 0. To compare differences in medians between matched pairs of cardiovascular values at t = 0 with t = 10, the Wilcoxon signed ranks test was used to account for repeated measurements over time in the same individual. The data distribution indicated that after t = 10, cardiovascular values tended to stabilize; thus, and to confirm that no further changes occurred after 10 min, we used linear regression (using ranks) with sampling points as fixed effects to formally assess changes on cardiovascular parameters after 10 min, using t = 10 as the reference value. To

Table 1. Distribution of cardiovascular parameters (median and interquartile range [IQR]) in six captive white rhinoceros ($n = 6$) for four treatments at sampling periods 0, 5, 10, 15, 20, and 25 min.

Arterial blood pressures	Treatment[a]	Time						
		0 min IQR[b]	5 min IQR	10 min IQR	15 min IQR	20 min IQR	25 min IQR	Overall IQR
Mean (mm Hg)	1	174 (155–187)	157 (143–169)	131 (129–144)	131 (121–142)	122 (112–129)	122 (112–129)	133 (124–150.5)
	2	111 (91–120)	93 (80–111)	92 (84–103)	93 (81–112)	90 (83–102)	94 (81–106)	94 (83–110.5)
	3	180 (171–188)	131 (110–136)	110 (96–116)	111 (92–122)	112 (95–128)	103 (90–114)	117 (99.5–142)
	4	131 (98–146)	81 (63–112)	77 (74–86)	80 (68–89	83 (72–87)	88 (77–97)	87 (72.5–99)
Systolic (mm Hg)	1	192 (175–209)	173 (164–190)	154 (146–158)	155 (141–162)	146 (134–152)	144 (135–155)	158 (144–167.5)
	2	123 (110–141)	112 (92–135)	112 (101–121)	112 (91–132)	110 (101–124)	114 (90–130)	114 (97.5–130.5)
	3	211 (196–226)	155 (130–173)	138 (121–144)	141 (113–148)	140 (119–157)	135 (113–140)	146 (124.5–179.5)
	4	145 (115–169)	101 (73–120)	100 (96–114)	104 (91–111)	102 (92–114)	110 (92–119)	106 (92–120)
Diastolic (mm Hg)	1	160 (136–169)	139 (129–151)	123 (107–129)	113 (103–125)	104 (97–111)	105 (94–112)	119 (104–132.5)
	2	97 (81–106)	76 (73–94)	78 (68–88)	81 (67–90)	75 (71–79)	79 (73–85)	79 (71.5–90)
	3	161 (151–166)	116 (93–121)	93 (82–99)	96 (79–106)	98 (78–112)	85 (72–97)	101 (83.5–124)
	4	117 (84–129)	67 (53–105)	65 (62–72)	67 (58–74)	72 (63–77)	76 (73–83)	74 (60–85)
Heart rate (beats/min)	1	136 (130–144)	141 (124–144)	134 (112–136)	125 (114–128)	120 (112–128)	114 (101–124)	127 (114–139)
	2	120 (112–132)	116 (104–124)	110 (92–116)	110 (92–116)	108 (88–116)	104 (84–112)	112 (96–120)
	3	139 (122–148)	89 (82–114)	65 (58–68)	63 (52–64)	60 (48–68)	68 (48–69)	68 (60–111)
	4	134 (114–140)	68 (58–80)	54 (46–56)	52 (45–56)	49 (48–56)	58 (53–61)	56 (48–69)

[a] Treatments: 1) etorphine; 2) etorphine plus azaperone; 3) etorphine and butorphanol i.v.; and 4) etorphine plus azaperone, and butorphanol i.v.

[b] IQR indicates interquartile range (25th to 75th percentile).

evaluate differences on cardiovascular parameters between treatment groups, we used linear regression (using ranks) to compare median cardiovascular parameters while adjusting for the effect of time, including sampling time points, as a fixed effect in the model. Statistical significance was set at $P < 0.05$ for all statistical tests.

RESULTS

Treatment 1: etorphine

At first sampling ($t = 0$), the median values for arterial blood pressures were: mean 174 mm Hg, systolic 192 mm Hg and diastolic 160 mm Hg (Table 1). These arterial pressures decreased significantly over time to 131 mm Hg ($P = 0.028$), 154 mm Hg ($P = 0.028$), and 123 mm Hg ($P = 0.028$), respectively, at $t = 10$. Between $t = 10$ and $t = 25$, mean and diastolic arterial blood pressures decreased further to 122 mm Hg ($P = 0.017$) and 105 mm Hg ($P = 0.012$), although there was no significant change in systolic blood pressure (Table 1; Fig. 1). Heart rate decreased from 136 beats/min to 114 beats/min over the immobilization period ($t = 0$ to $t = 25$); however, changes were not statistically significant between $t = 0$ and $t = 10$ ($P = 0.059$), and t = 10 and $t = 25$ ($P = 0.442$) (Table 1; Fig. 2).

Treatment 2: etorphine plus azaperone

At $t = 0$, the median values for arterial blood pressures in rhinoceros immobilized with etor-

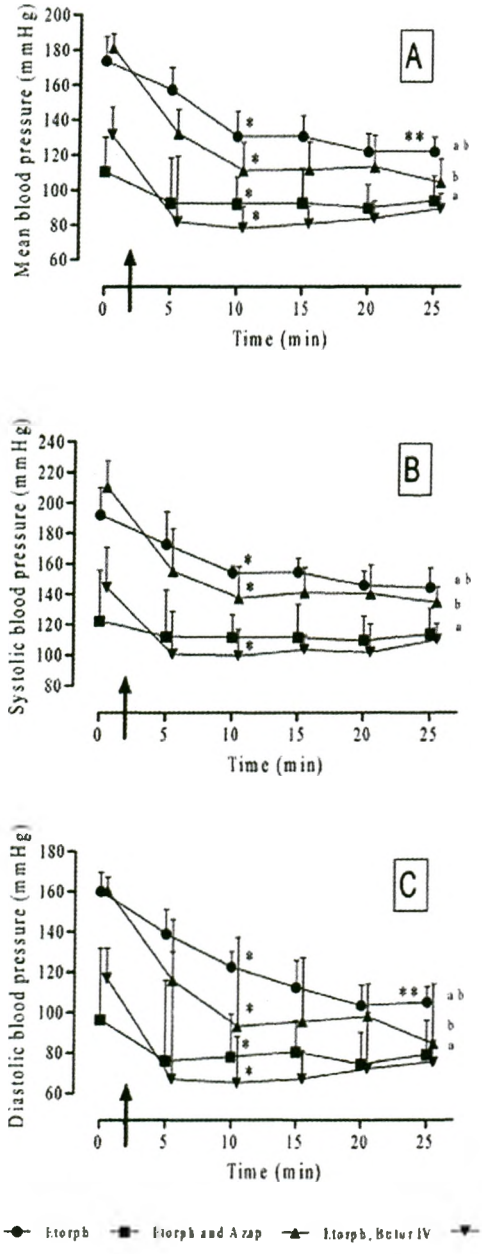


Figure 1. Arterial blood pressures: (A) Mean, (B) Systolic, (C) Diastolic. Note: Median and interquartile range of mean, systolic, and diastolic arterial blood pressures at sampling periods 0, 5, 10, 15, 20, and 25 min in six captive white rhinoceros ($n = 6$) for four treatments: 1) etorphine; 2) etorphine plus azaperone; 3) etorphine and butorphanol i.v.; 4) etorphine plus azaperone and butorphanol i.v. The black arrow indicates the time at which butorphanol was administered. *, indicates a significant ($P < 0.05$) difference within treatment between $t = 0$ and $t = 10$. **, indicates a significant ($P < 0.05$) difference within treatment between $t = 10$ and $t = 25$. a–b, the same letter indicates a significant ($P < 0.05$) difference in overall median values between treatments.

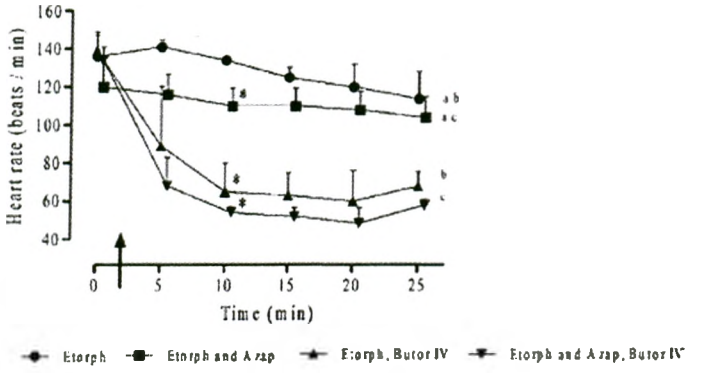


Figure 2. Heart rates. Note: Median and interquartile range of heart rates at sampling periods 0, 5, 10, 15, 20, and 25 min in six captive white rhinoceros ($n = 6$) for four treatments: 1) etorphine; 2) etorphine plus azaperone; 3) etorphine and butorphanol i.v.; 4) etorphine plus azaperone and butorphanol i.v. The black arrow indicates the time at which butorphanol was administered. *, indicates a significant ($P < 0.05$) difference within treatment between $t = 0$ and $t = 10$. **, indicates a significant ($P < 0.05$) difference within treatment between $t = 10$ and $t = 25$. a–c, the same letter indicates a significant ($P < 0.05$) difference in overall median values between treatments.

phine and azaperone were: mean 111 mm Hg, systolic 123 mm Hg, diastolic 97 mm Hg, and heart rate was 120 beats/min (Table 1). Significant decreases occurred between $t = 0$ and $t = 10$ in mean (111 mm Hg to 92 mm Hg, $P = 0.046$) and diastolic (97 mm Hg to 78 mm Hg, $P = 0.046$) arterial blood pressures; however, the decrease in systolic blood pressure (123 mm Hg to 112 mmHg) during the same period was not statistically significant ($P = 0.075$). Heart rate decreased significantly from 120 beats/min to 110 beats/min over the first 10 min of the study ($P = 0.028$) (Table 1; Figs. 1, 2). No further statistically significant changes were observed in mean, systolic, and diastolic arterial blood pressures and heart rate after 10 min.

Overall, during the 25-min immobilization period, arterial blood pressure measurements were significantly ($P < 0.001$) lower in rhinoceros immobilized with etorphine and azaperone compared with those that received only etorphine (differences in blood pressures were mean 39 mm Hg, systolic 45 mm Hg, and diastolic 40 mm Hg) (Table 1; Fig. 1). Median heart rates were also significantly different between the two treatments (15 beats/min, $P < 0.001$) (Table 1; Fig. 2).

Treatment 3: etorphine and butorphanol

After the administration of butorphanol i.v. ($t = 2$) in rhinoceros immobilized with etorphine, blood pressures decreased significantly ($P = 0.028$) between $t = 0$ and $t = 10$ (mean 180 mm Hg to 110 mm Hg, systolic 211 mm Hg to 138, and diastolic 161 mm Hg to 93 mm Hg (Table 1; Fig. 1). Heart rate also decreased significantly ($P = 0.028$) by 74 beats/min (139 beats/min to 65 beats/min) between $t = 0$ and $t = 10$. After $t = 10$, arterial blood pressures and heart rate did not change significantly over the rest of the immobilization period (Table 1; Fig. 2).

Generally and when controlling for the effect of time, cardiovascular parameters and heart rate were significantly lower in etorphine-immobilized rhinoceros that received butorphanol compared with those given sterile saline (decreases in arterial blood pressures were mean 15 mm Hg, $P = 0.001$; systolic 12.5 mm Hg, $P = 0.019$; and diastolic 18.5 mm Hg, $P < 0.001$; and heart rate 59 beats/min, $P < 0.001$) (Table 1; Figs. 1, 2).

Treatment 4: etorphine plus azaperone, and butorphanol

The administration of butorphanol at $t = 2$ to rhinoceros immobilized with a combination of etorphine and azaperone resulted in significant changes in arterial blood pressures between $t = 0$ to $t = 10$ (mean 131 mm Hg to 77 mm Hg, $P = 0.028$; systolic 145 mm Hg to 100 mm Hg, $P = 0.046$; diastolic 117 mm Hg to 65 mm Hg, $P = 0.028$). Between $t = 10$ and $t = 25$, there were no significant changes in blood pressures (Table 1; Fig. 1). Heart rate slowed significantly between $t = 0$ and $t = 10$ (134 beats/min to 54 beats/min, $P = 0.028$) and remained unchanged for the rest of the immobilization beyond $t = 10$ ($P = 0.442$) (Table 1; Fig. 2).

Overall in rhinoceros immobilized with etorphine and azaperone, there were no significant differences in arterial blood pressures between those administered i.v. butorphanol and those that did not receive butorphanol (Table 1; Fig. 1). Heart rate was significantly ($P < 0.001$) lower in rhinoceros administered butorphanol, compared with no butorphanol (56 beats/min compared with 112 beats/min) (Table 1; Fig. 2).

DISCUSSION

Immobilization of white rhinoceros with etorphine results in hypertension and tachycardia. The inclusion of azaperone with etorphine in the immobilizing drug combination reduces blood pressure associated with etorphine administration to values lower than those reported for unrestrained zoo animals.[6] Heart rates were also lower with this combination of opioid and tranquillizer compared with animals induced with only etorphine; however, the animals remained tachycardic with heart rates (≥100 beats/min) more than double those of normal values in unrestrained awake animals (32 to 42 beats/min).[6] Intravenous administration of butorphanol to rhinoceros immobilized with only etorphine resulted in some reduction in arterial blood pressure and no significant change in animals that were induced with both etorphine and azaperone. However, in both of these treatments, butorphanol administration resulted in significant reductions in heart rate.

The rhinoceros in this study, when immobilized with etorphine, were hypertensive at $t = 0$ (10 min after becoming immobilized) compared with standing unrestrained captive white rhinoceros (mean arterial blood pressure 173.5 mm Hg vs. 124 mm Hg, systolic 192 mm Hg vs. 160 mm Hg, diastolic 160 mm Hg vs. 104 mm Hg).[6] In addition, animals that received etorphine were tachycardic compared with standing unrestrained animals (heart rate 136 beats/min vs. 39 beats/min).[6] Hypertension and tachycardia have been previously reported in a small number of captive white rhinoceros immobilized with etorphine.[15,18] A mean intra-arterial blood pressure of 183 mm Hg was recorded in free-ranging animals chemically captured with an opioid combination of etorphine and fentanyl.[13] This blood pressure is similar to values of animals in the study, but the influences of combining fentanyl with etorphine or a potential adrenergic response induced by a chase and darting from a helicopter are unknown.[13]

The underlying mechanisms for increased arterial blood pressures and tachycardia in rhinoceros immobilized with etorphine are not fully understood. The cardiovascular response observed in the study may, in part, result from hypoxia caused by drug-induced respiratory depression, a common finding in immobilized white rhinoceros.[4,15] Hypoxia in humans resulted in increased heart rate, cardiac output, and systolic blood pressure, although mean and diastolic arterial pressures remained constant or fell slightly.[21] Hypoxemic activation of arterial chemoreceptors increases both sympathetic vasoconstrictor outflow to vascular beds and cardiac sympathetic activity increasing heart rate.[27] In domestic horses administered etorphine and acepromazine, a similar cardiovascular outcome was hypothesized to result from an etorphine-induced sympathetic response through the release of catecholamines from postganglionic neurons.[9,26] Cardiovascular pressor effects are consistent findings in opioid-immobilized perissodactyls, and have been reported in domestic and Mongolian horses, and Grevy's zebra.[12] Opioid receptors have been identified in rodent myocardial tissue preparations. If these myocardial receptors are ubiquitous in mammals, then activation of these receptors by etorphine could result in tachycardia with a potential increase in cardiac output.[11]

The initial hypertension in rhinoceros immobilized with etorphine had resolved at $t = 25$ to values similar to those in standing unrestrained zoo animals (arterial blood pressures were mean 122 mm Hg vs. 124 mm Hg, systolic 154 mm Hg vs. 160 mm Hg, and diastolic 105 mm Hg vs. 104 mm Hg).[6] Although the change in heart rate from 136 beats/min to 114 beats/min between $t = 0$ and $t = 25$ was not statistically significant, it may reflect a clinically relevant reduction in rate. However, rhinoceros remained tachycardic at $t = 25$ compared with unrestrained animals (heart rate 114 beats/min vs. 39 beats/min).[6] A decrease in blood pressures with a persistent tachycardia at the end of the immobilization is an unexpected result. A decreased drug effect due to redistribution and metabolism of etorphine would account for decreasing blood pressure over time but not the elevated heart rate. A possible explanation is an increase in heart rate to maintain cardiac output and arterial blood pressures in the presence of a reduced stroke volume and/or total peripheral resistance. In a recumbent immobilized animal, stroke volume may be reduced due to limited limb skeletal muscle activity causing blood pooling and decreased venous return to the

heart. Blood pooling may also occur due to an opioid-induced venous dilation, as has been reported in humans.[1] Respiratory depression and chest wall rigidity, which commonly occurs in immobilized rhinoceros, can also potentially decrease cardiac venous return by limiting the negative intrathoracic pressure that develops in association with inspiration and usually assists blood flow through the chest.[4,14,29] Severe hypoxia associated with opioid immobilization of rhinoceros may also be implicated in a local vasodilation in response to tissue hypoxia, a fundamental physiological response to ensure adequate oxygen supply-demand balance in metabolically active tissues.[14,31]

A persistent tachycardia with potential increased myocardial oxygen consumption is of clinical concern due to limited anaerobic capacity of the myocardium and pronounced hypoxia commonly associated with etorphine-induced respiratory depression.[15,30] It is also unknown how immobilizing drugs may influence the mechanisms that match coronary blood flow with myocardial oxygen requirements.[10] No obvious adverse effects associated with the persistent tachycardia were observed in the study animals; however, a negative outcome may occur in rhinoceros compromised due to age, disease, or poor nutrition.

Overall, arterial blood pressures in the study animals were significantly lower during the entire study period when azaperone was included with etorphine compared with etorphine only. At $t = 0$, rhinoceros had lower blood pressures compared with resting values in standing unrestrained captive animals. Heart rate decreased significantly over the first 10 min and was also clinically slower with the inclusion of azaperone; however rhinoceros were still tachycardic compared with values in standing resting animals (110 beats/min vs. 39 beats/min).[6] By comparison, Boardman et al. (2014) reported similar heart rates (118 beats/min), but higher blood pressures (systolic 162 mm Hg and diastolic 104 mm Hg) in white rhinoceros immobilized with similar doses of etorphine and azaperone.[2] Those rhinoceros were free-ranging and darted from a vehicle, which may have a caused a greater sympathetic response and hypertension than in the boma-acclimated rhinoceros.

Azaperone is advocated for rhinoceros immobilization to counteract etorphine-induced hypertension by antagonizing α_1-receptors in peripheral arterioles thus limiting vasoconstriction.[2,19,24] The hypotensive effects of azaperone have been described in domestic horses; azaperone can reduce mean arterial pressure by approximately a third from resting levels, for up to 4 hr.[20]

Potential mechanisms for tachycardia in etorphine/azaperone-immobilized rhinoceros could be related to a baroreceptor reflex induced by the hypotensive effects of azaperone, the sympathomimetic effects of etorphine and associated hypoxia, and possible direct opioid effects on the myocardium.[11,19,26] However, these explanations for the cardiovascular effects may be incomplete due to a limited understanding of receptor (opioid, dopamine, and adrenergic) distributions and associated functions within rhinoceros organ systems.[5,7]

While inclusion of azaperone reversed hypertension associated with etorphine immobilization, the lower than normal blood pressure that resulted in the rhinoceros also could be of clinical concern. A low mean arterial blood pressure can reduce blood flow through skeletal muscles, especially of the limbs, causing a buildup of metabolic waste products and a persistent hypoxia with possible myopathy and irreversible muscle damage.[25,29] Increased muscle activity from running prior to immobilization, and compression of muscles with occlusion of blood vessels in limbs positioned underneath a recumbent animal can further increase the risk of tissue injury.[25] A normal pressure response in other conscious megaherbivores is an increase in blood pressure when animals move from standing to lateral recumbency.[16] This compensatory response in the rhinoceros appeared to be prevented by the etorphine and azaperone combination.

Administering butorphanol to etorphine-immobilized rhinoceros resulted in rapid reductions in both arterial blood pressures and heart rate, reaching maximum changes within 10 min. Blood pressure values did not decrease to values observed in rhinoceros immobilized with etorphine and azaperone; however, heart rate was markedly slower in animals receiving butorphanol (65 beats/min compared with 110 beats/min at $t = 10$). In horses and humans, butorphanol administered alone did not significantly alter heart rate or blood pressure.[28,31] Due to a limited understanding of the complex pharmacology of butorphanol and its interactions with potent opioids like etorphine, it is difficult to definitively explain the mechanisms that result in these cardiovascular effects.[8,17] Etorphine is a potent pure opioid agonist at μ-, δ-, and κ-receptors, while butorphanol is a synthetic agonist–antagonist opioid that acts as a partial agonist at μ-, a pure agonist at κ-, and mainly as an antagonist at δ-receptors.[8,34]

Pharmacological responses resulting from butorphanol activity at μ-receptors frequently overshadow those of the κ-receptors.[8] Apart from the potential interactions of these two drugs at multiple opioid receptor types, pretreatment with etorphine can alter the binding of μ-receptors with other specific agonists.[8] It is therefore plausible that butorphanol's partial agonist effects on μ-receptors partly antagonize some of etorphine's μ-agonist effects, like tachycardia.

Butorphanol administration in etorphine- plus azaperone-immobilized rhinoceros did not alter blood pressures, but heart rates decreased to values clinically comparable to those recorded in unrestrained zoo rhinoceros.[6] Boardman et al. (2014) reported a decrease in both heart rate and blood pressure following the administration of butorphanol in rhinoceros immobilized with etorphine and azaperone.[2] The reason for the difference in blood pressure response is unknown, but may arise from the difference in the initial flight response in game-ranched animals darted from vehicles compared with boma-adapted rhinoceros darted from the ground.[2] Despite the difference in blood pressure responses between the two studies, rhinoceros in both groups had low blood pressures with a normal resting heart rate following butorphanol administration.

Since arterial blood pressures didn't change but heart rate slowed in etorphine- plus azaperone-immobilized rhinoceros following the administration of butorphanol, it is likely that the falling heart rate was associated with an increase in stroke volume, ejection fraction, or a combination of both to maintain cardiac output. Azaperone antagonizes peripheral vascular α_1-receptors so it is unlikely that an increased total peripheral resistance contributed to maintaining blood pressure.[19,29] The reduction in heart rate may result not only from a barometric response to an increase in cardiac output, but could also be related to a decrease in sympathetic response due to partial antagonism of etorphine's effects or an improvement in arterial oxygen tension. Buss et al. (2015) found that arterial oxygen partial pressures increased, from very low values, after the administration of butorphanol in boma-habituated rhinoceros immobilized with a combination of etorphine and azaperone.[4] A reduction in heart rate subsequent to improved blood oxygen levels would be expected if the original tachycardia was caused by hypoxia.[21]

Irrespective of immobilizing drug combination used, administration of butorphanol in boma-adapted white rhinoceros reduced heart rate. As previously mentioned, a potential benefit of decreased heart rate is a reduction in myocardial oxygen requirements in animals experiencing a marked hypoxia.

The mechanisms resulting in the cardiovascular changes recorded in this study can be elucidated further by developing a validated technique for determining cardiac output and through molecular investigation of drug-receptor interactions in rhinoceros. Cardiovascular changes associated with the use of etorphine, azaperone, and butorphanol should also be investigated in free-ranging rhinoceros as they are usually captured by darting from a helicopter, which induces a significant sympathetic response that may further alter cardiovascular function.[25]

CONCLUSION

Hypertension and tachycardia, which occurred in rhinoceros immobilized with etorphine, were reduced by both including azaperone in the immobilizing drug combination and administering butorphanol i.v. shortly after the animal had become recumbent. Inclusion of azaperone in the dart reduced etorphine-induced hypertension but did not correct tachycardia. Intravenous administration of butorphanol reduced the heart rate to values reported for resting unrestrained animals, but did not alter blood pressure further. This reduction may be due to an improvement in hypoxemia. A decreased heart rate may have a beneficial oxygen sparing effect on the myocardium. Similarly, a reduction in peripheral resistance and blood pressure by azaperone will reduce cardiac workload and hence oxygen requirements. However, of clinical concern, is that a profound decrease in blood pressure associated with the use of azaperone could cause adverse consequences due to reduced tissue perfusion, especially in compressed skeletal muscle groups in recumbent animals. Whether a lower azaperone dose than the one used may reverse the etorphine-induced hypertension without causing hypotension requires further investigation. In summary, azaperone reduced the hypertensive effects and butorphanol reduced the tachycardic effects of etorphine in immobilized white rhinoceros, thereby reducing potential risks associated with etorphine immobilization.

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CHAPTER 5

Discussion

White rhinoceros immobilization is a fundamental procedure used in the conservation of this endangered megaherbivore and allows for the capture, translocation and treatment of individuals. Immobilization also promotes scientific investigation which facilitates protection of this species. The potent opioids, including etorphine, are the only class of drugs which provide a rapid and reversible immobilization in rhinoceros (Haw *et al.* 2015). Opioids are essential in the capture of free-ranging rhinoceros as they allow for effective immobilizing-dose administration using low-volume darting systems (Burroughs *et al.* 2012a). However, immobilization is associated with significant changes in rhinoceros respiratory and cardiovascular physiology, which may result in high morbidity rates and mortalities (Boardman *et al.* 2014; Haw *et al.* 2014, Kock *et al.* 1995; Miller *et al.* 2013; Wenger *et al.* 2007).

The objectives of my studies were to investigate the cardiorespiratory pathophysiological effects of etorphine and azaperone, pharmacological agents most often used in white rhinoceros immobilization, and to examine the effectiveness of post-induction butorphanol IV in limiting these adverse effects. Reducing the risk of morbidity and mortality through an increased understanding and moderation of drug-induced cardiorespiratory changes in immobilized white rhinoceros will contribute to future successes in managing this species.

5.1 Respiratory effects in etorphine-immobilized white rhinoceros and the impact of post-induction butorphanol administration.

My studies described in chapters 2 & 3 confirmed that severe hypoxaemia and hypercapnia occur in white rhinoceros during immobilization with etorphine and azaperone. Similar changes in blood gases were also evident in rhinoceros immobilized with etorphine only. The initial, widely accepted, hypothesis was that opioids cause hypoventilation through depression of respiratory rhythm generating neurons, central and peripheral chemoreceptors, increased chest wall rigidity, and reduced airway patency. The resulting hypoventilation was suggested as the primary cause of the low oxygen and high carbon dioxide arterial pressures measured. However, the results of my study did not reveal a decrease in

ventilation as the primary cause of hypoxaemia but rather indentified increased metabolic oxygen consumption as a significant contributor in immobilized rhinoceros. Muscle tremors were positively associated with oxygen consumption and carbon dioxide production, leading to hypoxaemia and hypercapnia. Additionally, my results support the idea that abnormal blood gas values in immobilized-rhinoceros were also a consequence of an increase in the alveolar-arterial oxygen gradient.

My study results were consistent with literature reports that opioid-immobilized white rhinoceros experience life-threatening hypoxaemia and hypercapnia (Boardman *et al.* 2014; Haw *et al.* 2014; Kock *et al.* 1995; Miller *et al.* 2013; Wenger *et al.* 2007). However, early studies attributed these changes to hypopnea (Bush *et al.* 2004; Heard *et al.* 1992; Keep 1971; West 2008). Hypopnea leads to decreased minute ventilation, which has never been measured in immobilized white rhinoceros. Therefore, my results reported here are the first to show that hypoventilation does not predominantly contribute to the compromised blood gases observed in immobilized-rhinoceros. Respiratory minute ventilation at the first sampling period (10 minutes into the immobilization) was not significantly different from the expected value at rest, based on body mass. Although respiratory minute ventilation declined over the immobilization period, there were no significant changes in blood gases. Therefore, increased oxygen consumption and carbon dioxide production associated with an elevated metabolism is a more plausible explanation for the blood gas changes attributed to hypoventilation in earlier studies.

The idea that metabolic oxygen consumption and carbon dioxide production are increased in opioid-immobilized rhinoceros was first postulated by Miller *et al.* (2013) to explain arterial oxygen and carbon dioxide tensions, measured in field-immobilized white rhinoceros, which were not associated with hypoventilation. Oxygen consumption in our study animals was found to be inversely correlated with arterial oxygen partial pressure which, in turn, was significantly associated with increased muscle tremors. These results suggest that muscle tremoring in

etorphine-immobilized rhinoceros increased oxygen consumption through elevated metabolic activity and, consequently, increased carbon dioxide production. Although, not evaluated in my study, several reports suggest increased muscle tremoring is driven by a rise in plasma catecholamine levels associated with either a direct etorphine effect or opioid-induced hypoxia (Carter *et al.* 2002; Daniel & Ling 1972; De Lange *et al.* 2015; Schlarmann *et al.* 1973).

Another possible cause of observed hypoxaemia and hypercapnia in opioid immobilized rhinoceros was the increased $P(A\text{-}a)O_2$ gradient found in my studies (Chapter 2 & 3), as previously suggested by Wenger *et al.* (2007). Although the normal resting alveolar-arterial gradient is unknown for white rhinoceros, the value in our etorphine-immobilized rhinoceros was approximately four times higher when compared to a horse at rest (Doherty & Valverdeis 2008). The $P(A\text{-}a)O_2$ gradient is a non-specific measure of respiratory function and can be influenced by ventilation/perfusion mismatching, a physiological right-to-left shunt, or impaired alveoli–blood gas diffusion (West 2008). Studies in goats show that opioids cause pulmonary hypertension as a result of increased pulmonary vascular resistance (Meyer *et al.* 2015). Similar vascular changes in etorphine-immobilized rhinoceros may lead to an increased $P(A\text{-}a)O_2$ gradient, as observed in my study animals, due to hydrostatic fluid shifts resulting in pulmonary oedema or decreased pulmonary vascular red blood cell transit times, thereby reducing respiratory gas transfers. Further investigations are required to confirm the increased $P(A\text{-}a)O_2$ gradient in opioid-immobilized rhinoceros and determine the underlying cardiopulmonary physiological anomalies.

Apart from an increase in $P(A\text{-}a)O_2$ gradient and metabolic oxygen consumption in immobilized rhinoceros, our results indicate that the respiratory system's ability to respond to hypoxaemia and hypercapnia was inhibited. Initial PaO_2 (25 mm Hg) and $PaCO_2$ (76 mm Hg) values did not change significantly over the immobilization period in rhinoceros that only received etorphine and no butorphanol, although ventilation declined (Chapter 3). This result was unexpected as hypoxaemia and hypercapnia are drivers of increased respiration

(Pattinson 2008). There was also a 40% decline in oxygen consumption associated with a reduction in muscle tremoring by the end of the immobilization period. Assuming no other compensatory changes in respiratory function, this decrease in oxygen consumption should have resulted in an increase in PaO_2; however this increase was not observed. A possible explanation is that etorphine reduces chemosensory neuron and chemoreceptor excitability, and lowered the threshold at which hypoxaemia stimulates ventilation (Yaksh & Wallace 2011) to about 25 mm Hg. The normal threshold for rhinoceros at rest is unknown, but in humans, it is approximately 55 mm Hg (Kim *et al.* 2008). We propose that as oxygen consumption decreased over time, a PaO_2 value above 25 mm Hg would be associated with an etorphine-induced reduced hypoxic respiratory drive. With reduced stimulus, there would be a decrease in ventilation, as we observed, and arterial oxygen tension would remain static. Similarly, a $PaCO_2$ value above 70 mm Hg would not increase ventilation due to opioid chemoreceptor blockade. Opioids have been shown to block central chemosensitivity to hypercapnia and hypoxaemia (Koo & Eikermann 2011). Likewise, the response of peripheral carotid chemoreceptors to hypoxaemia, and possibly hypercapnia, would also be suppressed by opioids (McDonald & Lambert 2005; Pattinson 2008).

My results in chapter 2 & 3, suggest that azaperone causes limited respiratory effects when combined with etorphine to immobilize white rhinoceros. The PaO_2 and $PaCO_2$ in rhinoceros immobilized with etorphine or etorphine plus azaperone (prior to the administration of butorphanol) were very similar. Respiratory effects of azaperone are equivocal; increased and decreased respiratory rate in horses, improved ventilation in domestic animals and humans, and inhibition of opioid-induced respiratory depression have been reported (Lees & Serrano 1976; Radcliffe *et al.* 2000; Serrano & Lees 1976). It is possible that variable azaperone-associated respiratory outcomes are due to the distribution of dopamine receptor sub-types in respiratory regulatory structures, both centrally and peripherally (Lalley 2008; Tsuchiya *et al.* 2011). Azaperone binding at receptor types other than dopamine may also modulate respiration (Ahmed *et al.* 1980; Armtjo & Flórez 1974; Fryer & Jacoby 1998; Lemke 2007; Olson *et al.* 1979). Predicting

respiratory effects of azaperone in immobilized rhinoceros is further complicated by concurrent potent opioid respiratory influences. Additional studies are required to further investigate azaperone's respiratory effects in opioid-immobilized rhinoceros. Blood gases should be compared between etorphine and etorphine and azaperone immobilized-rhinoceros and the pathophysiology of differences investigated.

One drug that is increasingly being used in immobilized rhinoceros to purportedly improve ventilation is butorphanol. However, the reported effects on ventilation, reflected by changes in blood gas values, are variable and contradictory (Boardman *et al.* 2014; Haw *et al.* 2014; Miller *et al.* 2013; Wenger *et al.* 2007). These inconsistent findings were the impetus for my study to further investigate the cardiorespiratory effects of butorphanol.

In the study described in chapter 2, butorphanol (at 10 times the etorphine dose) administered IV to white rhinoceros immobilized with etorphine and azaperone resulted in improvements in PaO_2 and SaO_2, and no change to $PaCO_2$, at 10 and 20 min after administration. There were also improvements in the A-a gradient and arterial pH, and a decrease in respiratory rate. Despite the improvement in blood gases, the rhinoceros remained hypoxaemic and hypercapnic for the remainder of the 100 minute immobilization period. These results suggest that although butorphanol improved blood oxygenation, it was not due to improved ventilation as elevated arterial carbon dioxide levels remained unchanged. One of the explanations we proposed for improved oxygen values was a decrease in oxygen consumption which resulted in a relative improvement in PaO_2. A decrease in the initial moderate acidosis over time due to increases in base excess and bicarbonate ions, and a decrease in lactic acid, further supports our hypothesis that the changes in blood gases were influenced by metabolic changes. We also noted anecdotally in this study that muscle trembling and limb movements in the immobilized rhinoceros decreased following butorphanol administration, which may be associated with a change in metabolism. The design of this initial observational study, compared to the later randomized cross-over study, prevented

rigorous evaluation of butorphanol effects. However, the results highlighted the importance of further studies to investigate the causes of hypoxaemia and hypercapnia, and pathophysiological changes after butorphanol administration. The results also demonstrated that healthy white rhinoceros immobilized with etorphine and azaperone and administered a single dose of butorphanol, can tolerate prolonged periods of hypoxaemia and hypercapnia. This finding has had significant clinical consequences in managing free-ranging rhinoceros as it allows for prolonged immobilization and procedures not thought possible prior to this study.

In order to confirm that the blood gas changes observed in the initial study were a result of butorphanol administration and to elucidate the physiological mechanisms underlying these changes, we performed a cross-over study (chapter 3). We compared two interventions, etorphine-immobilization with post-induction butorphanol administration or etorphine with no butorphanol treatment, allowing us to rigorously evaluate butorphanol effects. Our results showed that butorphanol, administered IV, resulted in rapid, although limited, improvements in the markedly depressed oxygen and elevated carbon dioxide arterial tensions. However, rhinoceros remained hypoxaemic and hypercapnic over the entire immobilization period. Expired minute ventilation improved transiently for three minutes following butorphanol administration, then for the remainder of the study period, it was not significantly different from that in the rhinoceros that did not receive butorphanol. These findings support the hypothesis that butorphanol does not consistently change ventilation in etorphine-immobilized white rhinoceros. Other measured respiratory parameters, including $P(A\text{-}a)O_2$, were also not modified by butorphanol administration. However, butorphanol did result in a rapid decrease in muscle tremor scores. A significant inverse correlation between PaO_2 and $\dot{V}O_2$, a positive correlation between $PaCO_2$ and $\dot{V}CO_2$, and significant associations of both $\dot{V}O_2$ and $\dot{V}CO_2$ with muscle tremor scores support the theory that improvements in PaO_2 and $PaCO_2$ values were due to a reduction in metabolic activity.

Respiratory function depression is a significant adverse outcome in etorphine-immobilized white rhinoceroses and it has been proposed that butorphanol may limit this side-effect. Butorphanol has varied opioid receptor activities, including being an antagonist at MOR and agonist at KOR. It has been suggested that administration of butorphanol would limit etorphine-associated respiratory depression at MOR_2 without significantly reversing opioid-induced sedation in immobilized rhinoceros (McCrackin *et al.* 1994; Wenger *et al.* 2007; Yaksh & Wallace 2011). Anecdotal evidence from my own unpublished clinical work supports this hypothesis. However, variable respiratory outcomes have been reported in immobilized rhinoceros administered butorphanol (Boardman *et al.* 2014; Haw *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013; Van Zijll Langhout *et al.* 2016; Wenger *et al.* 2007). These different outcomes may arise from differences in immobilizing drug combinations, different butorphanol dosages, routes and times of administration, use of captive or free-ranging study animals, and results based on cross-over or observational studies (Boardman *et al.* 2014; Haw *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013; Van Zijll Langhout *et al.* 2016; Wenger *et al.* 2007). In studies by Wenger *et al.* (2007) and Miller *et al.* (2013), butorphanol was added to the immobilizing drug combination administered to free-ranging rhinoceros located and darted using a helicopter. Wenger *et al.* (2007) concluded that inclusion of butorphanol in the dart did not result in any observed respiratory benefits. The lack of effects may be explained by an inadequate butorphanol dose or the inclusion of detomidine as well as azaperone in etorphine-immobilizing combinations. Miller *et al.* (2013) reported that 78% of the rhinoceros receiving butorphanol in the dart remained standing and had higher PaO_2 and lower $PaCO_2$ compared to recumbent animals. This result suggests that butorphanol had an indirect respiratory benefit as immobilized rhinoceros tended to remain on their feet, a position which favours respiratory function compared to lateral recumbency (Morkel *et al.* 2010). However, results from this study did not support the hypothesis that the respiratory inhibitory effects of etorphine were antagonized by butorphanol at the opioid receptor level (Miller *et al.* 2013). Moreover, both of these studies were observational which limits the conclusion that can be made about individual drug effects (Miller *et al.*

2013; Wenger *et al.* 2007). In addition, drug dosages based on estimated body masses, variable drug delivery depending on the anatomical site and angle at which the dart struck the rhinoceros, gender differences including pregnancy status, and subjective distance estimates of distances traveled by a rhinoceros prior to and after darting may have affected results in these studies. The extent and variability of the adrenergic stress-response in rhinoceros associated with helicopter-darting, and how this response might influence drug effects and respiration were also not evaluated. These factors are all potential confounders, impacting results of field studies involving free-ranging rhinoceros located and darted using a helicopter.

As a result of the influence of multiple variables associated with field-immobilization, discussed above, it is difficult to definitively compare butorphanol-linked respiratory effects between studies. Two projects have reported arterial blood gas values prior to and following intravenous butorphanol administration in rhinoceros immobilized with etorphine plus azaperone (Boardman *et al.* 2014; Miller *et al.* 2013). Miller *et al.* (2013) found decreased $PaCO_2$ and no change in PaO_2 and suggested that these blood gas changes were linked to butorphanol-induced muscle relaxation. In contrast, findings of decreased $PaCO_2$ with increased PaO_2 supports the hypothesis that butorphanol antagonizes etorphine-induced ventilation depression in immobilized rhinoceros (Boardman *et al.* 2014).

My observational study results, described in chapter 2, in etorphine-azaperone immobilized boma-adapted rhinoceros administered butorphanol post-induction, suggested ventilation did not improve, since there was no change in $PaCO_2$, although PaO_2 increased. However, in a cross-over study performed in a similar controlled environment, the findings supported the improved ventilation hypothesis (Haw *et al.* 2014). It is difficult to explain why these two trials conducted under similar circumstances gave conflicting results. The different outcomes may be due to differences in age, gender and body mass between rhinoceros in my study compared to Haw *et al.* (2014). The dose of IV

butorphanol also differed between the two studies (15 times etorphine dose for Haw *et al.* (2014) and 10 times in my study). The differences in study outcomes highlight the influence that variation in study design and implementation methodology can have on interpreting effects of butorphanol administration on changes in ventilation or metabolic activity in immobilized rhinoceros.

In order to assess both respiratory and metabolic effects of butorphanol, we evaluated respiratory function and muscular activity in etorphine-immobilized boma-adapted rhinoceros. Azaperone was not included as an immobilizing drug as this eliminated another variable when investigating the interaction of the opioids. The study design incorporated the measurement of expired minute ventilation, mixed-expired and end-tidal carbon dioxide pressures, and expired and end-tidal oxygen fractions; it is the first time these variables had been measured in immobilized white rhinoceros. Using these parameters, we were able to calculate oxygen consumption as a measure of metabolic activity. Muscle tremoring, especially of limbs, was also scored as an indicator of activity. The results of this study supported the hypothesis that butorphanol increases PaO_2 and decreases $PaCO_2$ in immobilized rhinoceros through reducing metabolic oxygen consumption and carbon dioxide production. My findings also showed that rhinoceros remain severely hypoxaemic and hypercapnic, even though oxygen and carbon dioxide blood gas values were improved after butorphanol administration.

Oxygen insufflation in carfentanil-xylazine immobilized elk (*Cervus Canadensis manitobensis*) increased PaO_2 and reduced body and limb rigidity, suggesting that hypoxaemia contributed to muscle rigidity (Paterson, Caulkett & Woodbury 2009).These results support an alternative explanation for the improved blood gases and decrease in muscle tremoring observed in the etorphine-immobilized rhinoceros following butorphanol administration. The decrease in muscle activity may have been the result of an increase in arterial oxygen tension. Although, the rhinoceros did not receive supplementary oxygen as did the elk, there was an initial improvement in PaO_2 associated with a temporary increase in ventilation

immediately after the IV butorphanol was given. It is possible the acute increase in PaO_2 initiated a decrease in hypoxaemia-induced muscle tremors, which may have reduced oxygen consumption and carbon dioxide production.

In summary, my results support the concept that beneficial respiratory effects from butorphanol administration in etorphine-immobilized rhinoceros arise predominantly from a reduction in metabolic demands, not improvements in ventilation. These findings also offer an alternative explanation for blood gas changes reported by Haw *et al.* (2014) and Boardman *et al.* (2014) which were attributed to ventilation improvements. In my study, butorphanol IV resulted in an increase in PaO_2 and decrease in $PaCO_2$ due to an alteration in metabolism; however, these changes in both oxygen and carbon dioxide tensions mimic those associate with improved ventilation (West 2008).

Based on my findings, it is suggested that IV butorphanol administration improves the risk-profile and reduces the mortality potential associated with rhinoceros immobilization using opioids. Young healthy rhinoceros, as discussed above, appear to tolerate severe hypoxaemia and hypercapnia. However, butorphanol-associated improvements in arterial oxygen and carbon dioxide are expected to reduce the risk of immobilization-associated deaths, especially in compromised individuals. Although butorphanol does not return blood gas values to normal levels, it does increase PaO_2 by limiting skeletal muscle oxygen consumption, which makes more blood oxygen available for essential organ functions such as those in the brain and heart. Studies by Haw *et al.* (2014, 2015) indicated that the risks can be further reduced by intranasal oxygen insufflation in immobilized-rhinoceros following a single dose of butorphanol IV. Supplementary oxygen improves PaO_2 but does not influence $PaCO_2$, which continued to climb over time in the immobilized rhinoceros and potentially resulted in a worsening acidaemia. This risk may be higher in immobilized free-ranging rhinoceros which develop an initial metabolic acidosis due to physiological and psychological stress associated with dart-delivery from a helicopter (Haw *et al.* 2015).

Haw *et al.* (2014), reported unchanged low PaO_2 values over a 20 minute study period in etorphine-immobilized rhinoceros (no butorphanol was administered) under almost identical research conditions compared to my study. These authors also reported that critically low PaO_2 values did not improve with intranasal supplementary oxygen administration. This finding was unexpected as earlier reports indicated that tracheal oxygen insufflation in immobilized rhinoceros improved arterial oxygen tensions (Bush *et al.* 2004). Although ventilation was not measured (Haw *et al.* 2014), respiratory rates did decline in those immobilized rhinoceros administered oxygen. Proposed reasons for the adverse oxygen supplementation response included alveolar denitrogenation absorption atelectasis, intra-pulmonary shunt fractions caused by abdominal organ pressure on the lungs, and hypercapnia-induced central respiratory depression (Haw *et al.* 2014). My findings suggest an alternative explanation; supplementary oxygen may temporarily increase PaO_2 to above the decreased hypoxic threshold which was stimulating respiration, thereby removing the hypoxic ventilator drive. This effect would result in reduced respiratory minute ventilation associated with the decreased respiratory rate similar to what has been found in humans (Niesters *et al.* 2013).

5.2 Cardiovascular effects in etorphine-immobilized white rhinoceros and the impact of post-induction butorphanol administration

There is a paucity of information on the cardiovascular effects of etorphine administered on its own or with azaperone in immobilized rhinoceros. Therefore, we assessed these effects by measuring heart rate and arterial blood pressures in animals that had been given etorphine only and those administered etorphine with azaperone (Chapter 4). Rhinoceros immobilized with etorphine were initially tachycardic and hypertensive. The underlying mechanisms resulting in these outcomes were not investigated but a sympathetic pressor effect due to opioid-induced hypoxia or a direct stimulation of myocardial opioid receptors was proposed as the potential cause. Unexpectedly, during the immobilization period, blood pressures decreased to values observed in rhinoceros standing at rest,

although heart rate did not change significantly. A possible reason for heart rate not changing was a requirement to maintain cardiac output and arterial pressure due to a reduction in stroke volume and, or, total peripheral resistance. Rhinoceros immobilized with etorphine and azaperone had lower blood pressures than those in individuals administered only etorphine, and compared to standing awake rhinoceros. Heart rates, which were slower than those in etorphine-immobilized rhinoceros, were still considered tachycardic. We proposed that the arteriole α_1-blocking effect of azaperone may have countered etorphine-induced hypertension by preventing vasoconstriction and reducing peripheral resistance. We also suggested that the tachycardia was a baroreceptor response initiated by the lower blood pressure, sympathomimetic effects of etorphine, hypoxia, a direct myocardial opioid effect, or a combination of these factors.

My results, showing increased blood pressure and heart rate in etorphine-immobilized rhinoceros confirm earlier reports of etorphine-associated tachycardia and hypertension in captive rhinoceros, and tachycardia in wild-caught individuals (Heard *et al.* 1992; Kock *et al.* 1995; LeBlanc *et al.* 1987). The pathophysiological mechanisms resulting in these cardiovascular changes in rhinoceroses are unknown. Etorphine-induced circulatory system alterations vary between species; however, in perissodactyls (which include white rhinoceros), increased cardiac output, total peripheral resistance, and arterial blood pressure are consistent findings (Haigh 1982; Haigh 1990; Heard *et al.* 1992; Lees & Hillidge 1975). Proposed explanations for these cardiovascular outcomes include increased sympathetic activity and a response to opioid-induced hypoxaemia and hypercapnia (Heard *et al.* 1992; Kock *et al.* 1995; LeBlanc *et al.* 1987). Severe hypoxaemia and hypercapnia, commonly found in immobilized rhinoceros, may activate peripheral and possibly central chemoreceptors, increasing sympathetic heart and blood vessel stimulation, leading to a sympathetic chemoreflex (i.e., increased heart rate and blood pressure) (Guyenet 2006). It has been suggested that hypertension in carfentanil-xylazine immobilized bongo (*Tragelaphus eurycerus isaaci*) was as a result of increased blood nor-adrenalin concentrations caused by hypercapnia (Schumacher, Citino & Dawson 1997).

Etorphine may also have direct inotropic and chronotropic effects, depending on distribution of opioid receptor types within the rhinoceros myocardium (Headrick *et al.* 2012; Pugsley 2002, 2004; Yaksh & Wallace 2011). Additionally, etorphine may interact directly with myocardial cells and blood vessel smooth muscle, especially at higher doses, influencing ion-channel function and depolarization of these cells (Pugsley 2002, 2004). Opioid receptors within the sympathetic and parasympathetic nervous systems may also influence cardiovascular function (Feuerstein & Sirén 1987). In rats, MOR and DOR-specific agonists increase heart rate and blood pressure, most likely due to activation of the sympathoadrenamedullary axis (Feuerstein & Sirén 1987). Heart rate may also increase due to inhibition of vagal activity and baroreflex-mediated bradycardia, associated with either central or peripheral opioid effects (Headrick *et al.* 2012). Central MOR activation in rats has been shown to reduce baroreceptor control (Gordon 1986; Gordon 1990). Cardiovascular functions may also be changed by central opioid effects in immobilized rhinoceros; however, it is difficult to provide more definitive clarification since study results can be influenced by species, opioid type and site of administration (Feuerstein 1985; Feuerstein & Sirén 1987).

Moderation of blood pressure by azaperone in etorphine-immobilized megaherbivores is thought to be due to blocking at peripheral vascular α_1-receptors (Burroughs *et al.* 2012a). In horses, azaperone resulted in a decrease in total peripheral resistance and mean arterial blood pressure for up to four hours (Lees & Serrano 1976; Serrano & Lees 1976). However, azaperone also has antagonist activity at multiple additional receptors, including D_1- and D_2-like, 5-HT-, M_3- and H_1-receptors, which may influence cardiovascular outcomes, although our understanding of these effects is limited, especially in the presence of etorphine (Lemke 2007; Missale *et al.* 1998). Both D_1- and D_2-like receptors occur in the epicardium, myocardium, and endocardium of the human heart and exogenous dopamine administration increases cardiac contractility (Cavallotti *et al.* 2010; Pivonello *et al.* 2007). 5-HT-cardiomyocyte receptor activation has positive chronotropic and inotropic effects, and stimulation of blood vessel

receptors can result in either vasoconstriction or vasodilation, depending on the species (Ni & Watts 2006). Activation of H_1- and H_2-receptors results in vasoconstriction and vasodilation, respectively (Ebeigbe & Talabi, 2014). Stimulation of these histamine receptors in the heart can result in positive inotropic and chronotropic effects (Krzan 1996). Therefore, the observed cardiovascular effects of azaperone are the outcome of complex interactions with multiple receptors.

My results in chapter 4 show that tachycardia in rhinoceros immobilized with etorphine only and etorphine plus azaperone was reduced following administration of butorphanol IV. Butorphanol also reduced arterial blood pressures; however azaperone in the darting drug combination appeared to have a greater anti-etorphine-induced-hypertensive effect than did butorphanol in etorphine-immobilized rhinoceros. When butorphanol was administered to rhinoceros immobilized with etorphine plus azaperone, it did not significantly change arterial blood pressures, which were already lower than those in standing conscious rhinoceros. The cardiovascular effects attributed to butorphanol, observed in rhinoceros immobilized with etorphine plus azaperone, suggest that blood pressure was maintained by increased cardiac output rather than increased total peripheral resistance, since peripheral vasoconstriction is inhibited by the α_1-antagonist effects of azaperone. A number of scenarios can be proposed to explain the heart rate reduction following post-induction butorphanol administration. These include a baroreceptor response to increased cardiac output, decreased sympathetic response due to partial antagonism of etorphine effects, or an improvement in arterial oxygen tension associated with butorphanol administration.

My findings showed that hypertension was reduced by azaperone and tachycardia by butorphanol in etorphine-immobilized white rhinoceros. These findings are consistent with various reports of the cardiovascular effects following butorphanol administration in immobilized free-ranging rhinoceros, including a significant reduction in tachycardia and a lowering of blood pressure (Boardman *et al.* 2014;

Haw *et al.* 2015; Miller *et al.* 2013). However, my studies represent the first time that the cardiovascular effects of azaperone and butorphanol have been examined independently and in combination in etorphine-immobilized rhinoceros.

5.3 Clinical implications of findings

My research suggests that opioid-immobilized rhinoceros experience life-threatening side-effects, associated with low PaO_2 (25 to 31 mm Hg) and high $PaCO_2$ (65 to 76 mm Hg). Hypoxaemia in anaesthetized animals, with values less than 60 mm Hg, usually requires supportive interventions, and a hypercapnia greater than 70 mm Hg results in respiratory acidosis which can depress myocardial and CNS functions (Haw *et al.* 2014; Moens 2013; Read 2003). The young healthy rhinoceros used in our studies did not apparently experience short or long-term organ damage due to extremely low oxygen and high carbon dioxide arterial tensions resulting from immobilization. The reasons for these outcomes are unknown and may be related to the high oxygen affinity of rhinoceros haemoglobin and a lower tissue metabolic rate compared to smaller mammals (Baumann, Mazur & Braunitzer 1984; Heard *et al.* 1992). The degree to which rhinoceros can modulate neural breathing control may also play an essential role in individuals adapting to acute severe hypoxaemia and hypercapnia associated with opioid immobilization (Mitchell & Johnson 2003). Modulation of respiratory control is defined by Mitchell & Johnson (2003) as "neurochemically induced alteration in synaptic strength or cellular properties, adjusting or even transforming neural network function". My study animals were healthy sub-adult rhinoceros, a demographic which may have aided in compensating for severe physiological stresses, reducing potential negative outcomes. However, mortalities in rhinoceros due to immobilization have been reported (Kock *et al.* 1995), and, in my clinical experience, deaths can occur in immobilized free-ranging rhinoceros that do not receive adequate cardiorespiratory support and are compromised due to severe infections, poor nutritional status or advanced age.

Future studies should investigate the possibility of sub-clinical organ damage induced by hypoxaemia and, or, hypercapnia in immobilized rhinoceros. A

cardiac-specific troponin isoform (cTnI) is used in the detection of myocardial injury in humans (Babulin & Jaffe 2005). Central spinal fluid brain-specific creatine kinase isoenzyme and neuron-specific enolase are useful prognostic indicators of hypoxic brain injury (Kärkela, Bock & Kaukinen 1993). Serum creatine kinase isoenzyme (CK-MB and CK-MM) concentrations provide a measure of cardiac and, or, skeletal muscle injury (Apple 1999).

As a consequence of high morbidity rates from hypoxaemia and hypercapnia, and potential for mortalities, respiratory support interventions are required for opioid-immobilized free-ranging white rhinoceros. Providing intranasal supplementary oxygen has been proposed as a potential support strategy. However, giving additional oxygen can further compound high carbon dioxide blood levels without improving blood oxygenation in immobilized rhinoceros, unless an intravenous dose of butorphanol is administered prior to commencing insufflation (Haw *et al.* 2014). A combination of butorphanol, followed by oxygen administered intranasally, corrected hypoxia in immobilized rhinoceros, although the animals remained hypercapnic. The physiological changes induced by butorphanol which facilitated the increase in PaO_2 are not known and further studies are required to elucidate these. Bush *et al.* (2004) also advocated nasal tracheal insufflation as a means of improving safety for field-immobilized rhinoceros. They found that the technique improved PaO_2 and SaO_2 without changing $PaCO_2$. These results appear contradictory to those reported by Haw *et al.* (2014) since butorphanol was not administered to the rhinoceros. However, nalorphine (mixed opioid agonist-antagonist (Swan 1993)) and, or, doxapram (nonspecific respiratory stimulant (Swan 1993)) were given prior to or in conjunction with the supplementary oxygen. The outcomes of this study suggest that nalorphine and, or, doxapram may also facilitate an increase in PaO_2 during oxygen insufflation. As the study design used by Bush *et al.* (2004) was observational, it is not possible to confirm this recommendation based on the results provided.

My study in etorphine-azaperone-immobilized rhinoceros confirmed that young healthy rhinoceros can be kept safely immobilized for up to 100 min following a

single butorphanol IV dose (chapter 2). Prior to my study, the duration of white rhinoceros immobilization has been kept to a minimum to limit potential adverse effects associated with depressed respiratory function (Burroughs *et al.* 2012b). My results and subsequent clinical experience indicate that rhinoceros can be kept immobilized for extended periods to facilitate prolonged procedures including surgical treatment of snaring or bullet injuries resulting from poaching. Orphaned white rhinoceros calves immobilized with etorphine plus azaperone and administered a single butorphanol IV dose (5 to 10 times etorphine dose) have been safely airlifted for up to 50 min suspended by their feet under a helicopter. Free-ranging rhinoceros calves orphaned as a result of poaching also can now be recovered and stabilized with intravenous fluids administered for up to three hours using this immobilization regime.

My results have shown that muscle tremoring and limb shaking in etorphine-immobilized rhinoceros decreased rapidly (within three minutes) after butorphanol IV administration and was associated with an increase in PaO_2 and decrease in $PaCO_2$ (chapter 3). This observable change in muscle activity provides a mechanism by which butorphanol effects can be easily evaluated by a clinician. In immobilized wild-caught rhinoceros, it is costly and not always logistically practical to routinely monitor blood gases, and the value of using pulse oximetry, capnography, and other measures of blood gas levels still need to be validated in immobilized rhinoceros. The results from this study suggest an alternative indirect and subjective measure. Assessing the decrease in muscle tremoring following butorphanol IV administration may provide a measure of the improvements in arterial blood gases

The persistent tachycardia in rhinoceros immobilized with etorphine or etorphine plus azaperone raises concerns of possible hypoxic cardiac failure (chapter 4). As heart rate increases, there is a linear increase in myocardial oxygen consumption per beat and per minute (Boerth *et al.* 1969). There may also be a reduction in myocardial perfusion as heart rate increases (Mosley & Gunkel, 2007). Low arterial oxygen partial pressures are well documented in immobilized rhinoceros

(Buss *et al.* 2015). A combination of these factors may result in insufficient oxygen delivery to the myocardium to support aerobic ATP production required for contraction. In my study animals, there was no evidence of cardiac failure caused by hypoxia, although this may be due to limited cardiac functional assessment. However, the risk of cardiac failure may increase in rhinoceros compromised due to age, disease or poor nutrition. The potential for myocardial hypoxia also supports the administration of supplementary oxygen in immobilized rhinoceros as advocated by Haw *et al.* (2014 & 2015).

The decrease in heart rate following butorphanol administration provides a clinical measure of effect of this drug under field conditions. Since the dose of etorphine used to immobilize free-ranging rhinoceros is usually based on a combination of estimated body size and age, this estimate can result in significant variations in drug responses. Administering butorphanol to effect by monitoring the decrease in heart rate may permit tailoring of the dose for individual cardiovascular responses in etorphine-immobilized rhinoceros.

A word of caution: butorphanol administration in etorphine-immobilized rhinoceros results in an initial improvement in ventilation due to an increase in respiratory rate; however, this change in ventilation is transient and within a few minutes returns to pre-butorphanol levels. Therefore, to the casual observer, it may appear that butorphanol administration improves respiration; however, ventilation remains unchanged or progressively declines over time. Butorphanol also reduces the depth of immobilization in rhinoceros and an excess dose or incorrectly titrated doses may result in an animal becoming insufficiently immobilized and dangerous to personnel handling them.

5.4 Project limitations

As a consequence of the logistical challenges and welfare considerations associated with my research, small numbers of animals were used in each study. The small sample size likely results in greater variability in measured parameters which can influence detection of clinically important physiological changes using

statistical analyses. However, the use of a randomized crossover study design limited the influence of physiological variability between the individual rhinoceros and the outcome of comparisons between different drug combinations.

All of the rhinoceros used for these studies were boma-adapted and maintained under captive conditions for the duration of the research project. For the cross-over study, male rhinoceros of similar age and body mass were the study subjects. All animals were healthy and in apparently optimal body condition. Therefore, the impact of age, gender, nutritional status, and concurrent disease could not be evaluated. However, we suspect that the severe physiological changes observed in these healthy animals may lead to greater complications, and increase the risk of morbidity and mortality in compromised white rhinoceros.

In my studies, rhinoceros conditioned to captivity were used to limit the influence of potential confounders associated with immobilizing free-ranging animals. Rhinoceros captured in the field are typically approached and darted from a helicopter, which can influence arterial blood pressures, heart rate and metabolism as a result of increased physical exertion and an adrenergic stress response (Radcliffe & Morkel 2007). Age, body mass, and health status, which are usually unknown prior to darting in free-ranging rhinoceros, also influence cardiorespiratory responses to immobilizing drugs. Field drug dosages are based on an estimated body mass, and may vary significantly based on operator experience. All of these factors influence cardiorespiratory responses to drugs, limit comparison of results between studies, and understanding of underlying physiological mechanisms. Since physiological differences likely exist between free-ranging and captive rhinoceros, the results from this research may not be translatable to what occurs in field-immobilized white rhinoceros.

Although we were able to evaluate the effect of etorphine, azaperone, and butorphanol on blood pressures and heart rate in immobilized rhinoceros, due to technical limitations, it was not possible to evaluate changes in stoke volume or total peripheral resistance. The determination of cardiac output would allow for

the calculation of these cardiovascular parameters; however this measurement requires insertion of a catheter into the pulmonary artery, which is challenging due to the large body size, lack of easily accessible peripheral veins, and inadequate length of available catheters for rhinoceros. Inability to determine alveolar ventilation, cardiac output, pulmonary artery pressures, shunt fractions and ventilation-perfusion ratios, in part, limited a comprehensive understanding of physiological mechanisms influencing arterial blood gases and cardiorespiratory responses to these drugs.

5.5 Future research directions.

As discussed above, there are numerous factors that may impact physiological responses in field compared to boma-confined white rhinoceros. Haw *et al.* (2015) have indicated that respiration rate is higher and $PaCO_2$ lower in helicopter-darted immobilized free-ranging rhinoceros compared to boma-adapted individuals darted from foot with equivalent immobilizing-drug dosages. Our study results should be compared with those from field studies using similar drug combinations and doses. Pathophysiological influences of an increased sympathetic response, increased exertion and rise in body temperature associated with helicopter-based white rhinoceros immobilization could be evaluated with such studies.

Volumetric capnography should be used in the evaluation of immobilized-rhinoceros ventilation in the future. It will allow for the calculation and more comprehensive evaluation of respiratory dead space using either Bohr's formula or Enghoff's modification. Physiological dead space determination (chapter 3) required the collection and evaluation of mixed expired CO_2 of exhaled air collected in a Douglas bag. Physiological dead space was calculated using the Enghoff's modification of Bohr's formula (Tusman *et al.* 2012). Although dead space was determined relatively easily using this technique, it was not possible to determine alveolar deadspace ventilation as airway dead space ventilation was not known. Volumetric capnography has the potential to overcome this limitation (Tusman *et al.* 2012). As Enghoff's formula uses $PaCO_2$, it provides an overall measure of ventilation/perfusion mismatching and includes both wasted

ventilation (true dead space with alveoli of high ventilation/perfusion ratios) and wasted perfusion (shunt plus alveoli of low ventilation/perfusion ratios) (Tusman *et al.* 2012). Bohr's formula by comparison is considered a measure of wasted ventilation. Volume capnography allows for direct alveoli carbon dioxide tension determination which is required for Bohr's dead space calculation (Tusman *et al.* 2012).

The pulmonary and systemic circulatory structures are different in that they are considered low- and high-pressure systems, respectively. Pulmonary vascular resistance is low with little impedance of blood flow, even with increases in cardiac output and pulmonary artery pressures, which occurs in exercising animals (Robinson 2007). Although systemic arterial pressures were increased in my etorphine-immobilized study rhinoceros, pulmonary artery pressure effects are unknown and require further investigation. Meyer *et al.* (2015) have shown that etorphine-immobilized goats develop pulmonary hypertension which is thought to alter pulmonary gas exchanges as a result of interstitial and possibly alveolar oedema. The most likely cause of increased pulmonary hypertension was a pulmonary vasoconstriction and an increase in pulmonary vascular resistance following etorphine administration (Meyer *et al.* 2015). The increase in $P(A\text{-}a)O_2$ gradient observed in my study rhinoceros supports the idea that similar pulmonary vascular changes may occur in immobilized rhinoceros and requires further investigation.

Azaperone is frequently administered in combination with etorphine as it reduces induction time in white rhinoceros (Burroughs *et al.* 2012b). A number of investigators report that azaperone reduces opioid-associated hypertension and can result in normotension in immobilized free-ranging rhinoceros (Citino & Bush 2007; Bush *et al.* 2004; Hattingh *et al.* 1994). My study results in chapter 4 support the idea that azaperone reduces blood pressure in etorphine-immobilized rhinoceros. Arterial blood pressures were reduced to values lower than those recorded in captive rhinoceros standing at rest. This decrease in systemic blood pressure may have significant clinical implications in immobilized-rhinoceros as

the risk of compromised perfusion of oxygen-depleted dependent limbs in recumbent animals is possibly increased. Limited oxygen supply to and metabolic waste removal from limb skeletal muscle could result in a rhinoceros having difficulty rising from a recumbent to standing position. The capture of free-ranging rhinoceros usually involves partial etorphine-effects reversal using a mixed opioid agonist-antagonist so that the rhinoceros is able to rise to its feet and walk into a crate (Burroughs *et al.* 2012b). However, I have found that some etorphine/azaperone-immobilized white rhinoceros, especially large bulls, have difficulty standing due to an apparent proprioceptive deficit and limited weight-bearing capability of the dependent limbs. Cases have occurred in which immobilized rhinoceros are unable to stand on their hindlimbs following immobilization, despite the opioid effects being completely antagonized, and have subsequently required euthanasia. The importance of blood pressure effects of azaperone on limb perfusion in recumbent rhinoceros requires further investigation. A recommendation for reducing the azaperone dose in immobilization combinations should also be considered.

In my cardiovascular study, butorphanol decreased heart rate in boma-adapted rhinoceros immobilized with either etorphine or etorphine and azaperone which were initially tachycardic. Similar findings have been reported in free-ranging rhinoceros immobilized with etorphine plus azaperone that received butorphanol (Boardman *et al.* 2014; Haw *et al.* 2015; Miller *et al.* 2013). These findings suggest that etorphine, as the common factor between these various scenarios, is responsible for the tachycardia observed in the immobilized rhinoceros. However, further studies are required to test this hypothesis under different immobilizing conditions and with the inclusion of other drugs such as azaperone.

CHAPTER 6

Conclusions

My study results confirmed that the use of etorphine or etorphine with azaperone, results in severe hypoxaemia and hypercapnia in immobilized captive white rhinoceros. The severity of these abnormal blood gases normally requires cardiorespiratory support to prevent mortalities in anaesthetized domestic species. However, mortalities or clinically apparent organ damage did not occur in the healthy young adult white rhinoceros used in my studies. These results do not exclude the possibility of death in compromised rhinoceros or individuals immobilized under different circumstances.

Hypoxaemia and hypercapnia in etorphine-immobilized rhinoceros are not a consequence of reduced minute ventilation as has been proposed in previous studies. My findings suggest the primary cause is an increase in metabolic oxygen consumption and carbon dioxide production associated with increased muscle tremors. The cause of muscle tremors in immobilized rhinoceros is unknown but they likely result from direct or indirect sympathomimetic drug effects. The pathophysiology causing an increased calculated alveolar-arterial oxygen gradient seems to contribute to hypoxaemia in immobilized rhinoceros; however, the significance of and cardiopulmonary pathophysiology influencing the development of this gradient require further elucidation. Azaperone appears to have limited respiratory effects in immobilized rhinoceros but also requires more thorough investigation. Our studies suggest that although a decrease in minute ventilation may not be the fundamental cause of hypoxaemia and hypercapnia in etorphine-immobilized rhinoceros, low PaO_2 and high $PaCO_2$ also do not result in stimulation of ventilation as etorphine appears to reduce hypoxic and hypercapnic drive thresholds by depressing central and peripheral chemoreceptors.

Butorphanol administered post-induction in etorphine-immobilized rhinoceros resulted in moderate improvement in blood gases; although, hypoxaemia and hypercapnia persisted. My results support the idea that butorphanol reduces metabolic oxygen consumption rather than improving ventilation. Although hypoxaemia and hypercapnia persist following butorphanol administration, we believe its improvement in PaO_2 and availability of oxygen for essential organ

functions reduces hypoxic mortality risk in immobilized rhinoceros. This risk can be further mitigated by provision of intranasal supplementary oxygen (Haw *et al.* 2014). My results suggest rhinoceros that receive butorphanol post-induction can be kept immobilized for longer periods than was thought possible in the past. An increase in immobilization duration has clinical advantages in the treatment of injured rhinoceros and management of calves orphaned due to poaching.

Cardiovascular changes in our etorphine-immobilized rhinoceros included hypertension and tachycardia. Causes of these opioid-induced cardiovascular effects are unknown and may include direct myocardial drug effects, sympathetic system activation or a sympathetic chemoreflex due to opioid-induced hypoxia. A persistent hypertension and tachycardia in etorphine-immobilized rhinoceros raises concerns of potential increased cardiac workload and hypoxic cardiac failure. The inclusion of azaperone with etorphine in the immobilizing drug combination reduced blood pressure to below values for rhinoceros at rest; however, heart rate was still significantly elevated. The lower blood pressure in these individuals has been attributed by various authors to the α_1-blocking effects of azaperone. Azaperone is a multi-receptor antagonist and may influence blood pressure through activity at a variety of receptors. Low blood pressure associated with azaperone in immobilized rhinoceros can result in reduced dependent limb perfusion in recumbent animals. This concern should be further investigated and an azaperone dose reduction should be considered for the immobilization of rhinoceros.

Post-induction butorphanol administered IV reduced tachycardia, with a limited reduction in blood pressure in etorphine-immobilized rhinoceros. Butorphanol did not influence blood pressures but reduced tachycardia in individuals immobilized with etorphine plus azaperone. Determining cardiac output in immobilized rhinoceros would allow further investigation of these variable cardiovascular results. The reduction in tachycardia suggests butorphanol IV may have a myocardial oxygen sparing effect. A reduction in heart rate and limb tremoring in immobilized white rhinoceros provide subjective monitoring techniques by which

multiple butorphanol doses can be administered to achieve preferred cardiorespiratory outcomes.

My research has significantly advanced the understanding of the pathophysiological effects of immobilizing drugs and potential mechanisms that cause tachycardia, hypertension, hypoxaemia and hypercapnia in chemically-immobilized white rhinoceros. These findings have clinical applications in that they can be applied to reduce the morbidity and mortality risks associated with white rhinoceros immobilization, especially in compromised individuals. The study results also suggest that future research on interventions to counter opioid-induced respiratory depression and cardiovascular alterations should focus more on improving alveoli gas exchange and reducing the sympathomimetic and hypermetabolic effects of the potent opioids, rather than just focusing on improving ventilation and blood pressure.

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