REDUCTION OF RESPIRATORY DEPRESSION DURING CHEMICAL IMMOBILIZATION OF HERBIVORES

Anna Jean Haw

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, 2016

DECLARATION

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

I, Anna Jean Haw, 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.

_____

Anna Haw

Signed on the _____day of_____, 2016 in_____

ABSTRACT

Chemical capture using potent opioids is essential for the effective management and conservation of many wildlife species. Potent opioids are unparalleled in their ability to induce a rapid, reversible, catatonic immobilization. However, the beneficial effects of opioids are coupled with significant side-effects, most notably respiratory depression, to which white rhinoceros are particularly sensitive. Current treatments aimed at reversing opioid-induced respiratory depression (or respiratory compromise) have not been systematically compared. Moreover, the treatments do not appear to fully correct the hypoxaemia, hypercapnia and acidosis.

I therefore investigated and compared the effectiveness of current treatments, and also investigated the potential of a new treatment for opioid-induced respiratory depression. I conducted studies in goats and captive (boma-housed) white rhinoceros to reduce the effects of confounding variables that are present in field capture. After identifying the best protocol for reducing respiratory depression in captive rhinoceros, I determined whether that treatment was effective in field-immobilized rhinoceros.

In my first study, I compared the ability of two opioid agonist-antagonists to reverse etorphine-induced respiratory depression in immobilized goats by measuring respiratory parameters continuously, together with intermittent arterial blood gas measurements. I found that butorphanol more successfully reversed the etorphine-induced respiratory depression than did nalbuphine. In a controlled cross-over trial in boma-immobilized white rhinoceros, I then compared the efficacy of the treatments butorphanol, oxygen, and butorphanol combined with oxygen, in reversing etorphine-induced respiratory depression. Butorphanol (without oxygen) improved, but did not correct the hypoxaemia, hypercapnia and acidosis, while oxygen insufflation on its own exacerbated the hypercapnia and acidosis, and did not improve oxygenation. When butorphanol was combined with oxygen insufflation, the severe etorphine-induced hypoxaemia in immobilized white rhinoceros was completely corrected, however some hypercapnia and acidosis persisted.

Subsequently, I tested the butorphanol with oxygen treatment in field-immobilized rhinoceros. I found that the response to the helicopter chase altered the animal's physiology such that the high levels of inspired oxygen did not completely normalize arterial blood oxygen partial pressures. However, our treatment significantly improved arterial oxygenation

to near-normal partial pressures and reduced hypercapnia and acidaemia compared to pre-intervention values.

Finally, I assessed the cardiorespiratory effects of the ampakine, CX1942, in etorphine-immobilized goats to determine whether ampakines may offer advantages over currently available treatments in their ability to reverse opioid-induced respiratory depression. I demonstrated that the ampakine CX1942 improved arterial oxygenation and ventilation, without increasing arousal. Ampakines potentially offer advantages over doxapram, a conventional treatment, in reversing etorphine-induced respiratory depression in wildlife, but more species-specific studies are needed, and more water soluble and potent ampakines ultimately are required.

In summary, I recommend that to significantly reduce the risk of morbidity and mortality associated with respiratory depression during immobilization of wildlife, particularly white rhinoceros, stressful pursuits before darting should be minimized. Intravenous butorphanol should be administered, and oxygen insufflation initiated, as soon as the animal is recumbent. Potentially, the ampakine CX1942 may also be a successful treatment for opioid-induced respiratory depression, but further research is needed to ensure its use in rhinoceros and other wild animals is safe and effective. Ultimately, we should aim to prevent, rather than treat, opioid-induced respiratory depression in immobilized wildlife. In future, if more soluble and potent ampakines that can be combined with an opioid in an immobilizing dart are formulated, then the risk of opioid-induced respiratory depression could be reduced even further, if not negated.

ACKNOWLEDGEMENTS

Travelling the Ph.D. road within the Brain Function Research Group (BFRG) has been nothing short of incredible, and I am grateful to a number of people whom both consciously and unconsciously assisted me on my journey. First, and foremost, I would like to thank my enthusiastic supervisors, friends and colleagues, Andrea Fuller and Leith Meyer. It has been an honour to be their Ph.D. student. Leith and Andrea, who both delivered thoughtful, insightful feedback in record time, compelled me to improve my own academic ability. Particularly, I thank Andrea for her acute insight into animal physiology and her admirable scientific writing ability, and Leith for teaching me the art of arterial catheterization, among other unique veterinary skills. However, the support Andrea and Leith provided extended far beyond my academic and veterinary needs, and I am thankful for their holistic mentorship, which has enabled me to grow way beyond my Ph.D.

The BFRG, with its emphasis on collaboration, provided an excellent environment for intellectual curiosity, and I was fortunate to be handed unique opportunities within this Group. I thank particularly those members that assisted me with hours of goat trials, most notably, Zipho Zwane, along with many others that are too numerous to name, but whose contributions were significant. Members of the Central Animal Services (CAS) also provided essential help during my goat trials and I thank the whole CAS team, but particularly Nico Douths whom took great care of my goats and assisted in all my trials.

I am also indebted to the SANParks Veterinary Wildlife Services team at Skukuza, Kruger National Park. In particular, I thank the veterinarians; Markus Hofmeyr, Peter Buss and Michele Miller, and the expert capture team led by Marius Kruger. Thanks also goes to the veterinary technologists, Khosi Mathebula and Jenny Hofmeyr, for their time-keeping skills and data recording, and to Guy Hausler for field and laboratory assistance. I thank Johan Malan for coordinating the husbandry of the rhino during their prolonged stay in the bomas. I'm also grateful for the hours of helicopter time that Charles Thompson dedicated to my project. Without this well-oiled, efficient team, my rhinoceros work would not have been possible.

I thank Prof. John Greer from the University of Alberta, for his guidance with the ampakine study and Dr Jun Ren, Research Associate at the University of Alberta, for his advice and

help in acquiring CX1942. Thanks also to Dr. Arnold Lippa of Cortex Pharmaceuticals for generously providing CX1942.

A Ph.D. such as mine could not have been executed without substantial financial backing. Funding was provided by the National Research Foundation, South Africa, the Medical Faculty Research Endowment Fund and the University Research Committee, University of the Witwatersrand, and the South African Veterinary Association's Wildlife Group. The International Rhino Foundation, Disney's Animal Programs and Environmental Initiatives, SANParks and the Brain Function Research Group, University of the Witwatersrand, also provided substantial financial backing.

TABLE OF CONTENTS

		Page
DECLARATION……		II
ABSTRACT. …….		III
ACKNOWLEDGEMENTS……		V
TABLE OF CONTENTS……...		VII
LIST OF FIGURES……		IX
LIST OF TABLES……..		XII
AUTHOR CONTRIBUTIONS……..		XIII
LIST OF ABBREVIATIONS……		XV

CHAPTER 1

Introduction……		1
1.1	The history of chemical immobilization of wild animals……	2
1.2	The use of opioids in wildlife immobilization…….	4
1.3	Opioid receptors……...	7
1.4	Physiological effects of opioids……...	11
1.5	Opioid-induced respiratory depression……	14
1.5.1	Control of respiration……...	14
1.5.2	Opioids and respiration……	21
1.5.3	Opioid-induced respiratory depression in herbivores……..	22
1.5.4	Consequences of opioid-induced respiratory depression for animals..	23
1.6	Treatments for opioid-induced hypoxaemia……	25
1.6.1	Partial and full opioid antagonists……	25
1.6.2	Analeptics and chemoreceptor stimulants……	28
1.6.3	Drugs that increase cyclic adenosine monophosphate (cAMP)……...	30
1.6.4	Glutamate receptor modulators……	33
1.6.5	Microglial inhibitors…….	35
1.6.6	Increased inspired oxygen……	36
1.7	Summary and aims……..	37

CHAPTER 2

Nalbuphine and butorphanol reverse opioid-induced respiratory compromise but increase arousal in etorphine-immobilized goats (*Capra hircus*).
Veterinary Anaesthesia and Analgesia, in press doi:1111/vaa.12343, 2016…….. 59

CHAPTER 3

Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*).
BMC Veterinary Research 10:253, 2014…….. 70

CHAPTER 4

Improved cardiorespiratory function in immobilised wild white rhinoceros (*Ceratotherium simum*).
Journal of the South African Veterinary Association 86(1):E1-E10, 2015…… 80

CHAPTER 5

Ampakine CX1942 attenuates opioid-induced respiratory depression and corrects hypoxaemia in etorphine-immobilized goats (*Capra hircus*).
Veterinary Anaesthesia and Analgesia, in press, 2016…….. 91

CHAPTER 6

6.1 The opioid dilemma: nature of the problem…….. 115
6.2 Recommendations for solving the opioid dilemma…….. 120
6.3 Future directions…… 138
6.4 Conclusion……. 143

REFERENCES…….. 151

LIST OF FIGURES

CHAPTER 1

Fig. 1 Opioid receptor activation with consequent intracellular transduction pathways…….. 9

Fig. 2 Schematic representation of the ventral aspect of the brainstem, illustrating the respiratory control centre…… 17

Fig. 3 Neurotransmitters and other neuroactive agents involved in the central control of respiration..……... 19

CHAPTER 2

Fig. 1 Effects of intravenous butorphanol, nalbuphine and sterile water (control) on changes in respiratory rate (a), tidal volume (b) and ventilation (c) in etorphine-immobilized goats…… 64

Fig. 2 Effects of intravenous butorphanol, nalbuphine and sterile water (control) on changes in partial pressure of arterial oxygen (PaO_2) (a) and partial pressure of arterial carbon dioxide ($PaCO_2$) (b) in etorphine-immobilized goats……. 66

Fig. 3 Effects of intravenous butorphanol, nalbuphine and sterile water (control) on changes in oxygen consumption (a) and carbon dioxide production (b) in etorphine-immobilized goats……. 66

CHAPTER 3

Fig. 1 Respiratory rate (A), partial pressure of carbon dioxide (P_aCO_2) (B), partial pressure of arterial oxygen (P_aO_2) (C) and pH (D) of immobilized rhinoceros given different treatment interventions…….. 74

Fig. 2 Comparisons between the calculated values of the area under the response curves for respiratory rate (A), partial pressure of arterial carbon dioxide (P_aCO_2) (B), partial pressure of arterial oxygen (P_aO_2) (C) and pH (D) of immobilized rhinoceros given different treatment interventions……..... 75

CHAPTER 4

Fig. 1 Respiratory rate (a), partial pressure of carbon dioxide (P_aCO_2) (b), partial pressure of arterial oxygen (P_aO_2) (c) and pH (d) of field- and boma-immobilized white rhinoceros given intravenous butorphanol with oxygen insufflation..……... 85

Fig. 2 Bicarbonate concentrations in field-immobilized white rhinoceros given intravenous butorphanol with oxygen insufflation…… 86

Fig. 3 (a) Plasma lactate concentration of field-immobilized white rhinoceros given intravenous butorphanol with oxygen insufflation.
(b) Plasma lactate concentration at 5 minutes correlated with the estimated distance run during induction.
(c) Plasma lactate concentration at 5 minutes correlated with the average speed each rhinoceros ran during induction……. 86

Fig. 4 Heart rate and arterial blood pressure of immobilized white rhinoceros given intravenous butorphanol with oxygen insufflation…….. 87

CHAPTER 5

Fig. 1 Effects of intravenous doxapram, CX1942 and sterile water (control) on respiratory rate (A), tidal volume (B) and change in ventilation (C) in etorphine-immobilized goats…… 99

Fig. 2 Effects of intravenous doxapram, CX1942 and sterile water (control) on partial pressure of arterial oxygen (PaO_2) (A), haemoglobin oxygen saturation (SaO_2) (B), partial pressure of arterial carbon dioxide ($PaCO_2$) (C) and arterial pH (D) in etorphine-immobilized goats…… 102

Fig. 3 Immobilization scores in goats receiving intravenous doxapram, CX1942 and sterile (water)…… 104

CHAPTER 6

Fig. 6.1 Recorded numbers of rhinoceros poached in South Africa…… 116
Fig. 6.2 Turnover and number of game sold on auctions…… 117
Fig. 6.3 White rhinoceros immobilized in a boma…….. 126
Fig. 6.4 The effects of butorphanol, diprenorphine and oxygen in treating etorphine-induced respiratory compromise in field-immobilized white rhinoceros……... 131
Fig. 6.5 The effect on P_aO_2 following placement of the oxygen delivery tube in the trachea and nostril of immobilized white rhinoceros…….. 133
Fig. 6.6 Recommended immobilization protocol for white rhinoceros……... 134
Fig. 6.7 Oxygen cylinders in the field……. 140

LIST OF TABLES

CHAPTER 2

Table 1 Immobilization scores of the goats receiving butorphanol, nalbuphine and sterile water (control)……. 63
Table 2 Respiratory parameters of the goats receiving butorphanol, nalbuphine and sterile water (control) following etorphine-induced immobilization……. 65

CHAPTER 3

Table 1 Drug doses for white rhinoceros immobilization…….. 72

CHAPTER 5

Table 1 Scale used to score the level of immobilization in goats……..... 103
Table 2 Respiratory parameters of the goats receiving doxapram, CX1942 and sterile water (control) following etorphine-induced immobilization……. 105

CHAPTER 6

Table 6.1 Species most commonly traded at game auctions in South Africa and their sensitivity to opioid-induced respiratory depression…… 118

AUTHOR CONTRIBUTIONS

Chapter 2:

Haw A, Meyer LCR and Fuller A (2016) Nalbuphine and butorphanol reverse opioid-induced respiratory compromise but increase arousal in etorphine-immobilized goats (*Capra hircus*). *Veterinary Anaesthesia and Analgesia*, in press doi:1111/vaa.12343.

The idea of this study arose from discussions with my supervisors. I prepared the equipment, performed the experimental procedures, collected, analyzed and interpreted the data, and wrote the first draft of the manuscript. Andrea Fuller and Leith Meyer, my supervisors, helped with planning of the study, experiments, interpretation of the data, and edited the manuscript.

Chapter 3:

Haw A, Hofmeyr M, Fuller A, Buss P, Miller M, Fleming G and Meyer L (2014) Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*). *BMC Veterinary Research* 10:253.

The idea of the study arose from discussions with my co-authors. I prepared the equipment, collected, analyzed and interpreted the data, and wrote the first draft of the manuscript. Markus Hofmeyr was the driving force behind the initial idea of the study, and he performed the nasotracheal intubation, scored the level of immobilization and edited the manuscript. Peter Buss, Michele Miller and Gregory Fleming assisted with experimental work and edited the manuscript. Andrea Fuller and Leith Meyer, my supervisors, helped with planning of the study, experiments and interpretation of the data, and edited the manuscript.

Chapter 4:

Haw A, Hofmeyr M, Fuller A, Buss P, Miller M, Fleming G and Meyer L (2015) Improved cardiorespiratory function in immobilised wild white rhinoceros (*Ceratotherium simum*). *Journal of the South African Veterinary Association* 86:E1-E10.

The idea of the study arose from discussions with my co-authors. I prepared the equipment, collected, analyzed and interpreted the data, and wrote the first draft of the manuscript. Markus Hofmeyr assisted in experimental work, specifically with nasotracheal intubation and determining the immobilization score, and he edited the manuscript. Peter Buss, Michele Miller and Gregory Fleming assisted with experimental work and edited the manuscript.

Andrea Fuller and Leith Meyer helped with planning of the study, experiments and interpretation of the data, and edited the manuscript.

Chapter 5

Haw AJ, Meyer LCR, Greer JJ and Fuller A (2016) Ampakine CX1942 attenuates opioid-induced respiratory depression and corrects hypoxaemia in etorphine-immobilized goats (*Capra hircus*). In press, *Veterinary Anaesthesia and Analgesia*.

The idea of the study was my own. I prepared the equipment, collected, analyzed and interpreted the data, and wrote the first draft of the manuscript. John Greer assisted with planning the experiment, acquiring CX1942, and edited the manuscript. Andrea Fuller and Leith Meyer helped with planning of the study, experiments and interpretation of the data, and edited the manuscript.

As supervisors of the candidate, we confirm that Anna has described the roles of her co-authors accurately, and that she functioned as the principal investigator for all four studies.

Andrea Fuller: _____

Date: _____

Leith Meyer: _____

Date: _____

LIST OF ABBREVIATIONS

ACh	-	acetylecholine
Alt.	-	altitude
AMPA	-	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ANOVA	-	analysis of variance
ATP	-	adenosine triphosphate
°C	-	degrees Celsius
cAMP	-	cyclic adenosine monophosphate
cc	-	cubic centimetre
cm	-	centimetre
CO_2	-	carbon dioxide
δ	-	delta
DMSO	-	dimethyl sulphoxide
DRG	-	dorsal respiratory group
E	-	east
E_TCO_2	-	end-tidal carbon dioxide concentration
Fig.	-	figure
FiO_2	-	fractional inspired oxygen
f_R	-	respiratory rate
GABA	-	gamma aminobutyric acid
GPCR	-	G-protein coupled receptor
Gs	-	stimulating hetero-trimeric guanine-nucleotide-binding protein
I.M.	-	intramuscular
I.V.	-	intravenous
i.u.	-	international units
κ	-	kappa
kg	-	kilogram
kPa	-	kilopascal
L	-	litre
m	-	metre
m.a.s.l.	-	metres above sea level
mg	-	milligram
min	-	minute
ml	-	milliliter

mmHg	-	millimeters mercury
mmol	-	millimole
n	-	number
NMDA	-	N-methyl-D-aspartate
NTS	-	nucleus tractus solitarius
O_2	-	oxygen
od	-	outside diameter
$P(A\text{-}a)O_2$	-	alveolar-arterial oxygen partial pressure gradient
P_aCO_2	-	arterial partial pressure of carbon dioxide
P_aO_2	-	arterial partial pressure of oxygen
PDE4	-	phosphodiesterase 4
RTN/pFRG	-	retrotrapezoid nucleus/parafacial respiratory group
S	-	south
s	-	second
SANParks	-	South African National Parks
S_aO_2	-	arterial haemoglobin oxygen saturation
S_pO_2	-	peripheral capillary haemoglobin oxygen saturation
SD	-	standard deviation
SNK	-	Student-Newman-Keuls
TLR4	-	toll-like receptor 4
μ	-	mu
VCO_2	-	carbon dioxide production
VO_2	-	oxygen consumption
VRG	-	ventral respiratory group
vs.	-	versus
5-HT	-	5-hydroxytryptamine (serotonin)
%	-	percent

CHAPTER 1

Introduction

Southern Africa has a particularly rich and diverse wild animal population, but the effective management of these populations is becoming an increasingly critical and demanding task. Wildlife veterinarians within this region have pioneered many essential capture techniques, traditionally through trial and error and the necessity to solve conservation-related problems. Currently, a particular problem facing wildlife is the increasing pressure from illegal poaching. Rhinoceros species particularly are at the forefront of highly sophisticated poaching efforts as their horns are more valuable on the black market than are diamonds and cocaine. Although the white rhinoceros is the second largest land mammal, its size belies its physiological sensitivity, particularly to the respiratory depressant effects of potent opioids. Potent opioids are essential for the immobilization of large herbivores, but the very act of immobilization can place the animal at risk of morbidity and even mortality. Indeed, opioid-induced respiratory depression has been identified as the most significant risk in the immobilization of white rhinoceros (Burroughs et al. 2012a; Kock et al. 1995). As poaching continues to increase, the ability to safely immobilize rhinoceros and other large mammals becomes ever more important. It is therefore imperative that we find ways to mitigate the negative physiological consequences of capture and immobilization. This introductory chapter reviews the development of chemical immobilization in wildlife, with particular emphasis on the use of opioids in large herbivores and the efforts undertaken to combat opioid-induced respiratory depression.

1.1 The history of chemical immobilization of wild animals

For more than 10,000 years humans have employed various methods to capture large wild animals (Nielsen 1999). Hunting for consumption was the primary reason ancient humans caught wildlife. However, as civilizations developed, humans acquired a fascination and passion for possessing wild animals from exotic areas (Kisling 2001). Whether animals were hunted, used in circuses or kept for personal collections, egocentric reasons largely governed our need to capture wild animals (Nielsen 1999). Thus traditional capture methods, which relied heavily on physical restraint, were crude and harmful, often resulting in injury to, or death of the animal (Alford et al. 1974).

Today, chemical immobilization of wildlife has largely replaced various types of physical restraint. When used appropriately, chemical immobilization enables the safe restraint of

the largest species, while minimizing the stress and risk of injuries commonly associated with other methods. Conservation and management of wildlife has also replaced the early exploitative reasons for wildlife capture. In southern Africa, proclamation of the Kruger National Park in 1898 was a significant boost for wildlife protection. Unfortunately, by this time the former wide distribution of white rhinoceros throughout southern Africa had already been reduced to an isolated area in the Hluhluwe Game Reserve, where an estimated 20 white rhinoceros existed in total (Player 1993). The South African National Parks (SANParks) was then formed in 1926, with the responsibility to manage and protect South Africa's national parks and the wildlife within them. Integral to the management of these, and other parks, was the ability to safely capture wild animals for translocation, and later veterinary interventions, disease surveillance and identity marking. Immobilization, defined as the forced restriction of movement, therefore became an essential element of conservation wildlife management. In South Africa, Operation Rhino, in the early 1960s, was the first major wild mammal translocation effort. The successful rebound of the white rhinoceros population and the re-establishment of rhinoceros to former ranges could not have occurred without the use of projectile darts and immobilizing agents.

The first class of drugs successfully used for the chemical immobilization of wildlife via intramuscular injection was neuromuscular blocking agents, such as gallamine triethiodide (Swan 1993). These drugs paralyse muscles by affecting neurotransmission at the motor end-plate and therefore they do not have any effect on the central nervous system. An animal immobilized with a neuromuscular blocking agent will remain completely aware of its surroundings and will have normal perception of pain (Burroughs et al. 2012b). Gallamine triethiodide was experimentally used on white rhinoceros during Operation Rhino, but was quickly discontinued because of the narrow safety margin between doses that produced immobilization and those that produced death as a result of respiratory paralysis (Player 1967). Various other drugs and combinations of drugs, such as barbiturates, phencyclidine and phenothiazines, were tested on a trial and error basis, but chemical immobilization of herbivores largely remained a risky and difficult procedure (Harthoorn 1966). Barbiturates alone were not considered to be effective intramuscular immobilizing agents because of prolonged induction times, sometimes up to 1.5 hours (Savarie 1976). Phencyclidine, a dissociative anaesthetic, produced more promising results than the barbiturates, especially when combined with the phenothiazine, promazine. This combination was used to immobilize a number of different species,

including elephant, rhinoceros, hippopotamus, giraffe and buffalo, but the long recovery times were not ideal for field use (Savarie 1976).

It was not until wildlife veterinarians turned to one of the oldest class of drugs, opioids, that significant advances in wildlife immobilization started to take shape. As Sir Thomas Sydeman wrote in 1680: "*Among the remedies which it has pleased Almighty God to give to man to relieve his sufferings, none is so universal and so efficacious as opium*" (Gustein & Akil 2001). Indeed, the derivatives of opium have relieved the sufferings not only of man, but also of many captured wild animals, as these opium-related drugs have enabled rapid, effective and reversible immobilization of the largest land mammals.

1.2 The use of opioids in wildlife immobilization

Opium, first cultivated around 3400 BC from the seedpods of the poppy, *Papever somniferum,* has been exploited for its analgesic and euphoric properties for thousands of years (Holden et al. 2005; Rosenblum et al. 2008; Sharma et al. 2015; Zöllner & Stein 2006). However, it was only in about 1817, and after some near-fatal experiments, that the active ingredient and standard exogenous opioid analgesic, morphine, was isolated from opium by the German pharmacist, Sertürner (Huxtable & Schwarz 2001). Since the isolation of morphine, many new, semi-synthetic and synthetic opioids have been developed. Today, morphine and other opioid analgesics remain the most important drugs for moderate to severe pain management (Dahan et al. 2010; Montandon et al. 2011).

Although opioids are best known for their potent analgesic effects, a number of other responses, including catatonia, are elicited by opioid receptor activation, but the exact nature of these responses vary among species. Opioid-induced immobilization is most commonly carried out in species belonging to the orders Artiodactyla, Perissodactyla and Proboscidea (Haigh 1990), as opioids generally induce a quick catatonic immobilization without causing severe excitement or respiratory depression in most species within these orders. In other orders, such as Felidae and Primates, opioids may induce catatonia, but are also commonly associated with excessive excitement or severe central nervous system depression (Swan 1993).

Diethyl-thiambutene was the first synthetic opioid exclusively used for the capture of African ungulates (Savarie 1976). Morphine, combined with chlorpromazine (a phenothiazine) and hyoscine was the initial drug combination used to translocate white rhinoceros (Player 1967), but diethyl-thiambutene was thought to offer advantages over morphine due to its increased solubility (Haigh 1990). However, despite the improved solubility, volumes of up to 20 ml were needed to induce immobilization in rhinoceros (Harthoorn 1966) and the large darts often left extensive, suppurating wounds in the animals (Player 1967). Large, heavy darts will have greater impact energy than smaller, lighter darts, thus causing more tissue damage and inflammation thereby increasing the risk of developing dart-site wounds. The large volume of injected solution also limited the darting range and resulted in slow absorption so that after darting it would take up to 20 minutes before the rhinoceros became immobile, in which time it could travel up to five miles (Player 1967). Another disadvantage of diethyl-thiambutene is that it had to be dissolved in sterile solution just before use (Haigh 1990). As a result of these disadvantages, diethyl-thiambutene is no longer available.

In 1963, another semi-synthetic opioid called etorphine was introduced, which revolutionized wildlife management (Harthoorn & Bligh 1965). Etorphine is produced from the opium alkaloid thebaine, and has a pharmacological profile similar to that of morphine (Harthoorn 1966). The essential difference between morphine and etorphine is that etorphine has a much higher affinity for opioid receptors and is highly lipophilic, enabling rapid penetration of the blood-brain barrier (Haigh 1990). Consequently, the effective immobilization potency of etorphine is reported to be about 1000 times that of morphine (Harthoorn 1966). Therefore a small injection volume of etorphine, capable of fitting in a 3 cc dart, can bring about a rapid immobilization in large herbivores. The quick induction results in less stress for the animals and the smaller darts reduce the risk of severe abscesses. Shortly after the introduction of etorphine, and its specific antagonist diprenorphine, these drugs were tested in a variety of wildlife species and etorphine was shown to cause rapid immobilization, sedation, analgesia and muscle relaxation (Alford et al. 1974). However, responses varied among species and side-effects were commonly noticed in free-ranging mammals (Alford et al. 1974). Etorphine also significantly simplified the capture process during Operation Rhino. It was primarily through the introduction of etorphine that hundreds of white rhinoceros were translocated to parks throughout South Africa during Operation Rhino, thereby saving the species from

extinction. Today, as poaching levels increase exponentially, rhinoceros species are yet again facing the risk of extinction. Etorphine remains central to the implementation of more advanced management and conservation strategies such as dehorning, international translocations and sophisticated veterinary procedures for the treatment of wounded animals.

The advent of potent opioids, such as etorphine, carfentanil and thiafentanil, has facilitated wildlife work far beyond rhinoceros translocations. Etorphine is a semi-synthetic opioid and worlwide, it is the most common opioid agent used for the immobilization of ungulates (Schumacher 2008). Carfentanil is a synthetic opioid that is more potent, and has a longer duration of action than that of etorphine, but it is not available as a registered product in southern Africa (Burroughs et al. 2012b). Thiafentanil is a newer synthetic potent opioid derived from the opioid analgesic fentanyl. Thiafentanil, developed specifically for wildlife work, is available in southern Africa and has a potency similar to that or etorphine, but a quicker onset of action (Burroughs et al. 2012b; Schumacher 2008). Many biologists and veterinarians rely on potent opioids to carry out advanced procedures in a variety of free-ranging wild animal populations (Haigh 1990). These drugs have also improved the ability of zoo veterinarians to manage their captive wild animals. In southern Africa, where wildlife and ecotourism are an important, large and growing sector of the economy, potent opioids such as etorphine remain central to the conservation of wildlife, and indeed the growth of the wildlife industry as a whole.

Although significant advances have been made towards the improvement of wildlife immobilization protocols, morbidity and mortality directly related to the capture event still occur (Arnemo et al. 2006; Kock et al. 2005; Meltzer & Kock 2012). Indeed, in compromised animals, such as those wounded from poaching incidents, acute death during immobilization is even more common (Buss, personal communication). Also, sophisticated physiological monitoring equipment has revealed that immobilized animals are often more physiologically compromised than previously thought, which may lead to undetected chronic complications (Fahlman 2008). Thus it is imperative to refine and improve immobilization protocols, especially in the most vulnerable and sensitive species, such as white rhinoceros.

1.3 Opioid receptors

Opioid drugs such as morphine and etorphine exert their effects by mimicking the endogenous opioid peptides, the natural ligands for opioid receptors. The endogenous opioid system is complex and widespread throughout the nervous system, playing a particularly important role in pain relief and stress regulation. However, the endogenous opioid system is also fundamental in the modulation of many other physiological processes (Dahan 2007; Holden et al. 2005). The exact physiological roles of endogenous opioid peptides are not completely understood but they appear to function as neurotransmitters, neuromodulators, and in some cases as neurohormones (Inturrisi 2002).

These endogenous opioid peptides are divided into three main groups namely, the endorphins, enkephalins and dynorphins, and they act on three classes of receptors termed the mu-opioid receptor (MOP), delta-opioid receptor (DOP) and kappa-opioid receptor (KOP) (Gutstein & Akil 2001). There are considerable structural similarities amongst these three groups of endogenous opioid peptides, and each family demonstrates variable affinities for mu-, delta-, and kappa-receptors, with none of them binding exclusively to a single opioid receptor (Grim & Lamont 2007). A fourth endogenous opioid, the nociception/orphanin FQ peptide also exists, but it does not act on the classic opioid receptors (mu-, delta-, and kappa-opioid receptors) but rather on its own receptor, called the nociceptin orphanin FQ peptide receptor (NOP) (Gutstein & Akil 2001; Holden et al. 2005; McDonald & Lambert 2005). Other opioid receptors have been proposed (e.g. sigma, epsilon and orphanin) but are currently not considered as "classical" opioid receptors (Kieffer & Gavèriaux-Ruff 2002).

Etorphine, the most commonly used exogenous opioid drug for wildlife immobilization, is categorized as a full opioid agonist, meaning it has agonistic activity at the mu-, kappa- and delta-opioid receptors. On the other hand, naloxone and naltrexone are full opioid antagonists, thus they occupy the opioid receptors but do not elicit a response. Mixed opioid agonist-antagonists, such as butorphanol, nalorphine, nalbuphine and diprenorphine have the ability to display agonistic activities at some opioid receptors, and antagonistic activities at others.

Each of these opioid receptors, on which these endogenous and exogenous opioids act, belongs to the large family of G-protein coupled receptors (GPCRs) (Shang & Filizola 2015). Like all GPCRs, each opioid receptor consists of an extracellular N-terminus, seven transmembrane helices, three extra- and intracellular loops, and an intracellular C-terminus (McDonald & Lambert 2005). When an opioid binds to its receptor, it activates the G-protein, which leads to: (i) inhibition of adenylyl cyclase thus reducing the levels of cyclic adenosine monophosphate (cAMP); (ii) activation of potassium channels resulting in potassium efflux; and (iii) closing of voltage-gated calcium channels (Haji et al. 2000; Inturrisi 2002; McDonald & Lambert 2005; Pattinson 2008) (Fig 1). At the pre-synaptic level, decreased calcium influx results in reduced release of neurotransmitters, thereby inhibiting synaptic transmission (Grimm & Lamont 2007). Post-synaptically, enhanced potassium efflux leads to neuronal hyperpolarization, resulting in reduced transmission of nerve impulses (McDonald & Lambert 2005; Pattinson 2008). The ultimate result, therefore, of opioid receptor activation, is a decrease in neuronal excitability. Opioids may also inhibit GABAergic transmission in a local circuit, for example in the brainstem (Inturrisi 2002). As each opioid peptide and opioid receptor has a distinct genetic differentiation, many researchers proposed that each receptor type should mediate a distinct profile of pharmacological actions (Shook et al. 1990; Zöllner & Stein 2006).

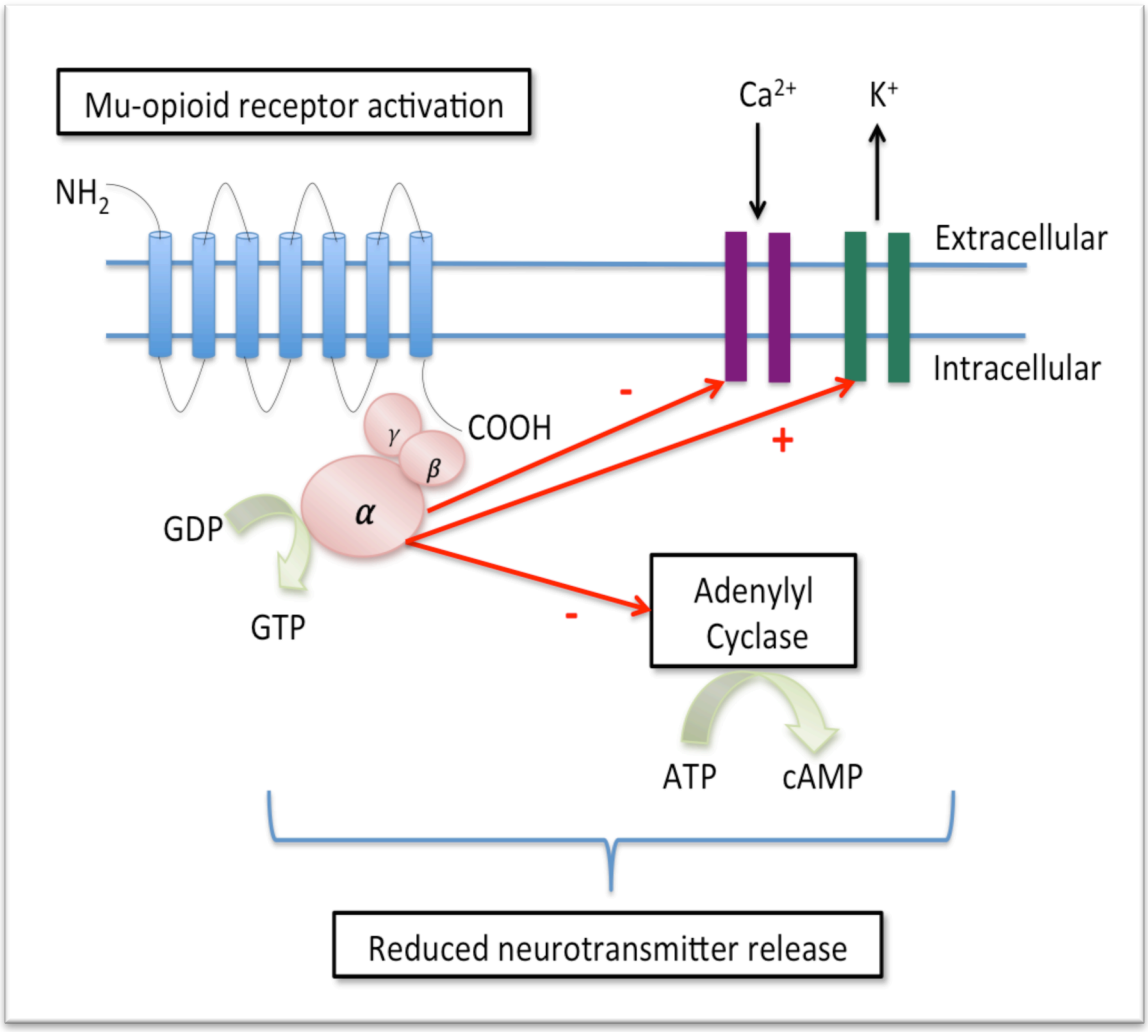


Figure 1. Opioid receptor activation. Seven transmembrane structure of an opioid G-protein-coupled receptor. Receptor activation by opioid ligands leads to initiation of intracellular transduction pathways that include stimulation of potassium efflux, inhibition of voltage-sensitive calcium channels and inhibition of adenylyl cyclase. Figure redrawn from McDonald J & Lambert DG (2005) Opioid receptors. *Continuing Education in Anaesthesia, Critical Care & Pain* 5:22-25.

However, despite years of research, it remains considerably difficult to clearly separate the pharmacological actions following the activation of different opioid receptors. This difficulty is due, in part, to the substantial variability in the affinity and intrinsic activity of both endogenous and exogenous opioid ligands for different opioid receptors. Because of the considerable structural homology among the three classically described opioid receptors, it is likely that there are significant interactions among these receptors in different tissues (Grimm & Lamont 2007). A given opioid drug may interact to a variable extent with all or some of the opioid receptors, and may act as either an agonist, as a partial agonist or partial antagonist, or as a full antagonist (Swan 1993). Partial agonists have low intrinsic activities and therefore activate G-proteins less efficiently, resulting in a lower overall activation at full receptor occupancy compared to full agonists (Hoskins & Hanks 1991; Shang & Filizola 2015). Pure antagonists occupy, but do not activate the receptor and therefore they do not elicit a response. Also, most opioid drugs interact with more than one receptor and may produce effects through each of these receptors that can be additive, synergistic, or antagonistic to each other (Shook et al. 1990). The term agonist-antagonist refers to the opioids that behave as agonists, partial agonists or antagonists at different opioid receptors (Hoskins & Hanks 1991). The response elicited by these mixed drugs also may differ with the concentration of the administered drugs, as a higher concentration will increase the interaction with receptors of lesser affinity (Shook et al. 1990). Moreover, partial agonists act as agonists when given alone, but will act as an antagonist when given with another agonist of higher affinity (Shook et al. 1990). The pharmacodynamic responses to opioid stimulation, therefore, depend on the nature of the receptor and the affinity and efficacy of the opioid for that receptor, as well as the dose of the administered opioid (Boom et al. 2012).

To add to the complexity of opioid pharmacology, recent knowledge of the crystal structures of opioid receptors reveals that these receptors are dynamic systems and that opioid ligands can activate multiple intracellular signaling pathways (Shang & Filizola 2015). Therefore, while our knowledge of opioid receptors and their effects upon activation has greatly improved over the years, opioid pharmacology remains a complicated science where many questions remain to be answered. Broadly, mu-receptor activation is primarily responsible for analgesia, respiratory depression, catalepsy and euphoria, while kappa-receptor activation conveys analgesia and sedation, with some degree of respiratory depression (Bowdle 1998; Kieffer 1999). The exact functional role

of delta receptors is not well understood, but substantial parallels exist between mu- and delta-receptors (Gutstein & Akil 2001). Although the nociceptin orphonin FQ peptide receptor is similar in structure to the mu-, delta- and kappa-opioid receptors, the pharmacology of the receptor is different and its exact physiological role is unclear, although it appears to be involved in nociception (Bowdle 1998; Grimm & Lamont 2007). In general, it is accepted that the mu-receptor mediates most of the clinically relevant analgesic effects, but also most of the adverse side effects, including respiratory depression (Dahan & Teppema 2003; Grimm & Lamont 2007). Earlier reports suggested different mu-receptor subtypes, with mu_1-receptors mediating analgesia, and mu_2-receptors responsible for respiratory depression (Ling et al. 1985). However, molecular studies have now confirmed that only a single mu-receptor gene exists (Pasternak & Pan 2011). The variability in pharmacological responses to opioid receptor binding may result from extensive alternative splicing of the mu-opioid receptor, such that a number of splice variants that lack certain amino acid sequences are generated (Pasternak 2014; Zöllner & Stein 2006).

Since the opioid receptors are widespread and implicated in many physiological processes such as pain and stress regulation, immune-responsiveness and the control of breathing (Dahan 2007; Inoue et al. 1998; Zubieta et al. 2001), exogenous opioid drugs also have multiple effects (Boom et al. 2012; Dahan 2007). Although the exact mechanism of some of these opioid-induced responses are not well understood, they generally occur as a result of opioid receptor activation, which leads to decreased neuronal excitability within specific anatomical sites.

1.4 Physiological effects of opioids

Traditionally, opioids were explored for their potent analgesic effects, and today morphine remains the gold standard for the treatment of postoperative pain (Boom et al. 2012). The analgesic effects of opioids are primarily centrally mediated and arise from their ability to inhibit transmission of nociceptive information from the spinal cord dorsal horn (Grimm & Lamont 2007). Opioid analgesics also control descending pain circuits that originate from the midbrain (Grimm & Lamont 2007). The interactions between opioid receptors and various spinal and supraspinal locations are complex and not fully understood (Grimm & Lamont 2007). Also, although opioids were traditionally considered as centrally acting

analgesics, we now know that opioids can also produce measurable analgesia by activation of opioid receptors in the peripheral nervous system (Stein et al. 2001).

Although opioids are used primarily for their analgesic properties, they also induce a multitude of other effects on a variety of body systems. Indeed, the adverse opioid responses often limit the use of high-doses of opioids, thereby reducing their analgesic efficacy.

A common consequence of acute high-dose opioid administration is intense, generalized muscle rigidity and myoclonic movement (Benthuysen et al. 1986; Bowdle 1998; Vankova et al. 1996). While human anaesthesiologists fear this adverse opioid effect, the opioid-induced muscle rigidity aids in rapid immobilization of wildlife species. However, severe muscle rigidity also has negative consequences for immobilized wildlife. As in humans, opioid-induced chest and abdominal rigidity decreases the ability of immobilized animals to adequately expand the thoracic cavity for breathing. Moreover, generalized muscle rigidity causes increased muscle metabolism, which leads to increased oxygen consumption (Benthuysen et al. 1986). Muscle rigidity also compromises blood flow to the muscles thereby limiting oxygen supply and predisposing to anaerobic metabolism, which contributes to generalized acidaemia (Benthuysen et al. 1986). Like opioid-induced analgesia, muscle rigidity is the result primarily of activation of central mu-opioid receptors (Vankova et al. 1996). Thus opioids with very fast equilibrium between blood and brain are more likely to produce rigidity because of relatively high peak brain concentrations following systemic administration (Bowdle 1998).

Together with rigidity, the decreased neuronal excitability induced by opioids also causes sedation and drowsiness. The sedative effects of opioids are beneficial for both wildlife immobilization and for human anaesthetic induction, but severe sedation in the post-operative setting can be problematic (Inturrisi 2002). Another central effect of opioid analgesics is their action on the medullary chemoreceptor trigger zone, which produces nausea and vomiting (Inturrisi 2002). However, there appears to be a lot of individual variation in the degree to which opioids induce emesis and the exact mechanism is not fully understood (Bowdle 1998).

Other central effects of opioids, which are concerning for human clinicians, is their ability to induce euphoria and a psychological and physical dependence. This opioid dependence is the basis behind heroin and other opioid drug addictions. Indeed, fear of addiction is a major concern limiting the use of appropriate doses of opioids in hospitalized patients in need of analgesia (Inturrisi 2002).

Although the majority of the most profound opioid effects are centrally mediated, one of the most common side-effects of opioid analgesics is constipation, which is primarily peripherally mediated (Bowdle 1998; Inturrisi 2002). Opioid drugs act at multiple sites in the gastrointestinal tract and spinal cord to produce a decrease in intestinal secretions and motility, resulting in a dry stool and constipation (Inturrisi 2002).

Surprisingly, most opioids have minor effects on cardiac output, cardiac rhythm and arterial blood pressure (Grimm & Lamont 2007). For this reason, opioid drugs are commonly used in anaesthetic protocols for patients with pre-existing cardiac disease. However, rapid administration of morphine and pethidine is known to potentially cause histamine release, which causes hypotension in humans (Bowdle 1998). Other opioids belonging to the fentanyl group such as fentanyl, sufentanil, alfentanil and remifentanil do not release histamine in humans, but typically reduce heart rate by a vagomimetic action that can be antagonized by atropine (Bowdle 1998). Conversely, etorphine has been found to induce hypertension in immobilized wildlife presumably via activation of the sympathetic nervous system (Roquebert & Delgoulet 1988), which leads to systemic and pulmonary vasoconstriction (Haigh 1990; Heard et al. 1992; Heard et al. 1996). Thus the cardiovascular effects of opioids may vary according to the dose and type of opioid used, the background physiological state of the animal and species-specific variations.

One effect of opioids that is not so well elucidated is their role in the immune system. As opioid binding sites have been identified in microglial cells, Kupffer cells, astrocytes, peripheral blood monocytes and macrophages (Dobrenis et al. 1995), opioids are thought to play some role in the immune system (Stefano et al. 1993). Indeed, *in vitro* assays and animal studies suggest that opioids such as morphine can suppress a number of immunological variables (Gutstein & Akil 2001).

Opioids therefore, together with their unparalleled analgesic effects, cause an array of adverse consequences, but the most dangerous and life-threatening of these side-effects is respiratory depression (Bowdle 1998).

1.5 Opioid-induced respiratory depression

Potent opioids have revolutionized wildlife immobilization and opioids are the most commonly used drugs in the treatment of moderate to severe pain in humans. However, in both human anaesthesia and wildlife immobilization, opioid-induced respiratory depression is the adverse effect most feared by medical and veterinary clinicians (Burroughs et al. 2012b; Zöllner & Stein 2006). Death from opioid overdose is nearly always a result of respiratory arrest (Gutstein & Akil 2001). Morphine-induced human fatalities started occurring soon after the drug was first isolated (Dahan et al. 2010) and since the early 1990s deaths from unintentional opioid overdoses in the United States of America have been rising steeply (Okie 2010). This rise of opioid-related deaths has paralleled efforts aimed at encouraging clinicians to become more proactive in identifying and treating chronic pain (Okie 2010). In 2010 over 16,000 people in the United States of America died from prescription potent opioids (Volkow et al. 2014). As the number of opioid-related human deaths continues to rise, it is evident that solutions to opioid-induced respiratory depression not only are important for wildlife, but also essential for the advancement of human medicine. In order to understand how opioids affect respiration and therefore how opioid-induced respiratory depression can be treated, it is important to understand how breathing and respiration is controlled under normal circumstances.

1.5.1 Control of respiration

Respiration is the process in living organisms whereby energy is produced from the oxidation of complex organic substances leaving carbon dioxide as a by-product. Oxygen intake and carbon dioxide removal are therefore essential for energy production and thus the survival of many living organisms. Breathing, a remarkably complex behaviour in vertebrates, regulates the oxygen uptake and carbon dioxide removal in the lungs. Breathing patterns, therefore, adapt according to metabolic demand, changes in blood pH and, in non-primate mammals, body temperature (Feldman & Del Negro 2006).

In mammals there are three basic elements to the respiratory control system, namely: (i) sensors, which gather information and feed it to (ii) a central controller in the brain, which coordinates the information and, in turn, sends impulses to (iii) effectors (respiratory muscles) which cause the breathing motions (West 2012). The rhythmic discharges from the brain that produce breathing motions are regulated by alterations in arterial oxygen, carbon dioxide and pH, and this chemical control of breathing is supplemented by a number of nonchemical influences (Lumb 2005a). The breathing cycle itself can be divided in three phases, namely inspiration, post-inspiration (passive expiration) and late expiration (active expiration) (Bianchi & Gestreau 2009). During inspiration, the flow of air into the lungs is controlled by both pump and resistance muscles (Feldman & Del Negro 2006). The diaphragm, unique to mammals, is the principal inspiratory pump muscle along with the external intercostal muscles, while muscles of the mouth, nose and throat, as well as the smooth muscles of the bronchi control airway resistance (Feldman & Del Negro 2006). These upper airway muscles are innervated by glossopharyngeal (IX), vagal (X) and hypoglossal (XII) cranial nerves, while the phrenic nerve and intercostal motor neurons control the diaphragm and intercostal muscles, respectively (Greer et al. 1991). During normal breathing cycles, the expiratory motor outputs are active during late expiration, although the entire phase of expiration may be passive in quiet breathing (Bianchi & Gestreau 2009).

The continuous rhythmic breathing activity originates in the respiratory centre, situated in the medulla (Fig. 2). Within this highly complex central control system, kernels of respiratory neurons, which possess intrinsic bursting properties (Schwarzacher et al. 1995; Smith et al. 1991), are coupled with a number of modulatory inputs such that this rhythmic activity is highly adaptable to changing respiratory requirements. The respiratory centre is bilaterally organized into dorsal and ventral groups of respiratory neurons situated in the bulbar brainstem, from the rostral pons to the caudal border of the medulla (Richter & Spyer 2001). The ventral respiratory column, which is divided into several functional compartments including the retrotrapezoid nucleus/parafacial respiratory group (RTN/pFRG), Bötzinger complex, pre-Bötzinger complex and rostral and ventral respiratory groups (Spyer & Gourine 2009), is essential and sufficient to generate the respiratory rhythm. The region termed the retrotrapezoid nucleus/parafacial respiratory group was first thought to be two distinct anatomical regions, but it is now understood to be the same region, which is referred to as the parafacial respiratory group in perinatal

animals, and the retrotrapezoid nucleus in adults (Smith et al. 2013). For the purpose of this thesis, I will therefore refer to this anatomical region as the retrotrapezoid nucleus.

The retrotrapezoid nucleus is one of two coupled, anatomically distinct, intrinsic rhythm generators, with the other being the pre-Bötzinger complex (Feldman et al. 2003; Feldman & Del Negro 2006; Mellen et al. 2003; Smith et al. 1991). Expiratory neuron bursts are proposed to originate from the retrotrapezoid nucleus (Feldman & Del Negro 2006), while inspiratory drive in generated by the pre-Bötzinger complex (Janczewski & Feldman 2006; Ramirez & Richter 1996; Richter et al. 2000; Smith et al. 1991). Under normal conditions, the pre-Bötzinger complex is the dominant of the two oscillators and, indeed, under resting conditions, when there is no active expiration, the pre-Bötzinger complex may be the only anatomical site from which respiratory rhythm is generated (Feldman & Del Negro 2006).

The other functional compartments of the ventral respiratory column are involved in shaping the basic rhythm and relaying this pattern to respiratory motor neurons (Smith et al. 2007). The rostral and ventral respiratory groups, located in the caudal regions of this column, have a concentration of inspiratory and expiratory premotor neurons (Feldman & Del Negro 2006). Neurons in the rostral ventral respiratory group are predominantly responsible for relaying inspiratory drive to phrenic motor neurons innervating the diaphragm, while the caudal ventral respiratory group contains expiratory neurons (Smith et al. 2013). The more rostrally located Bötzinger complex contains predominantly expiratory inhibitory interneurons and provides the inspiratory-expiratory phase alternation during normal breathing (Feldman & Del Negro 2006; Smith et al. 2013).

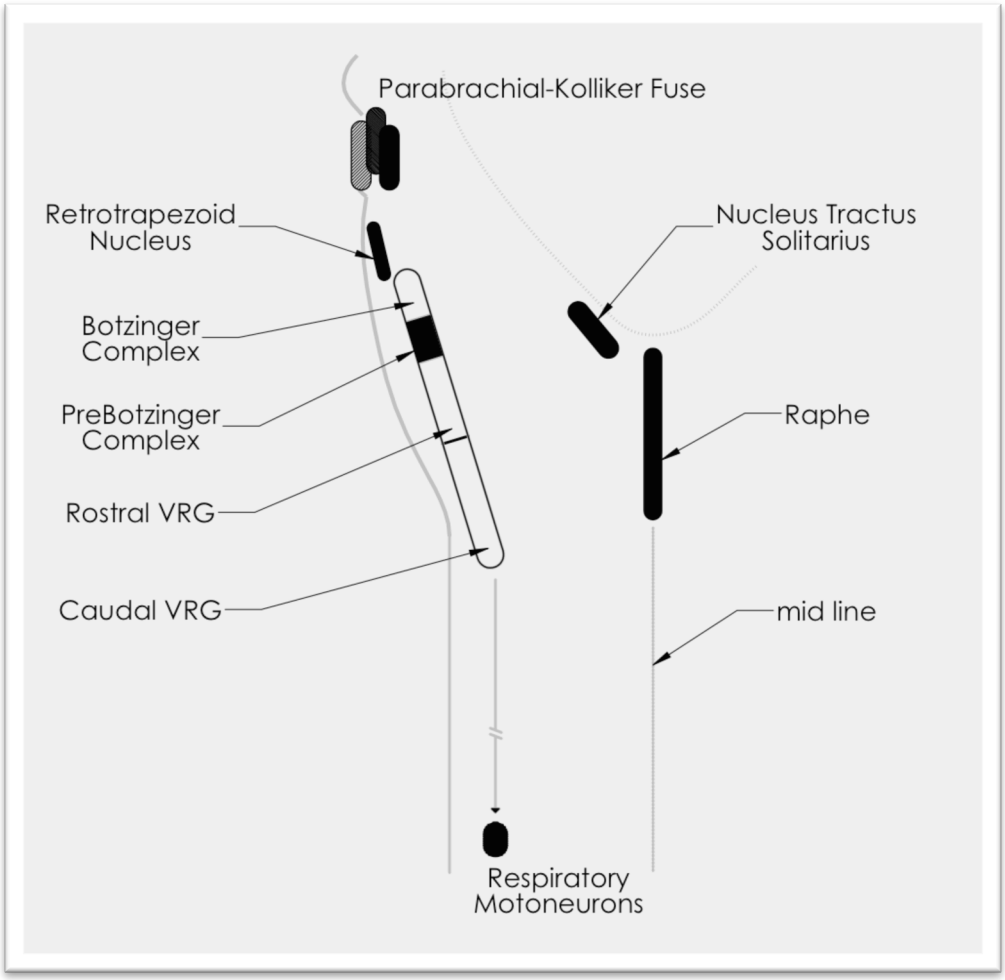


Figure 2. Schematic representation of the ventral aspect of the brainstem, illustrating the respiratory control centre. Central chemoreceptors are situated in the retrotrapezoid nucleus and midline raphe nuclei. These chemoreceptors activate premotor neurons of the ventral respiratory group (VRG) that includes the pre-Bötzinger complex. Sensory input from the peripheral chemoreceptors activates the nucleus tractus solitaries, which also projects to the VRG. Signals from the VRG are relayed to respiratory motoneurons in the spinal cord and phrenic nucleus. The Parabrachial-Kolliker Fuse also contains respiratory neurons that are primarily implicated in volume control.

Diagram redrawn from: Dahan A, Aarts L & Smith TW (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 112:226-38.

The respiratory rhythm is therefore initiated and shaped by a complex network of both excitatory and inhibitory neurons, which are activated by various neurotransmitters (Fig. 3). Glutamate, the primary excitatory neurotransmitter in the mammalian nervous system, activates excitatory pathways through two ionotropic glutamate receptors, namely the N-methyl-D-aspartate (NMDA) receptors and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. AMPA receptor activation is important for inspiration, while the NMDA-mediated mechanisms are important for phase transition from inspiration to expiration (Haji et al. 2000). GABA and glycine are the neurotransmitters primarily responsible for the inhibition of respiratory-related neurons (Haji et al. 2000). Other neurotransmitters, such as adenosine, serotonin, dopamine, substance P and acetylecholine play a neuromodulatory role, together with endogenous opioids (Burton & Kazemi 2000). Endogenous opioids do not appear to play a critical role in the generation of normal respiration (Bonham 1995; Santiago & Edelman 1985), but opioid receptors are abundantly expressed throughout the central respiratory network, particularly in the ventral respiratory group (Lonergan et al. 2003; Pattinson 2008). The abundance of opioid receptors and endogenous opioids within the central respiratory network suggests that endogenous opioids play an important tonic role in the regulation of breathing (Santiago & Edelman 1985).

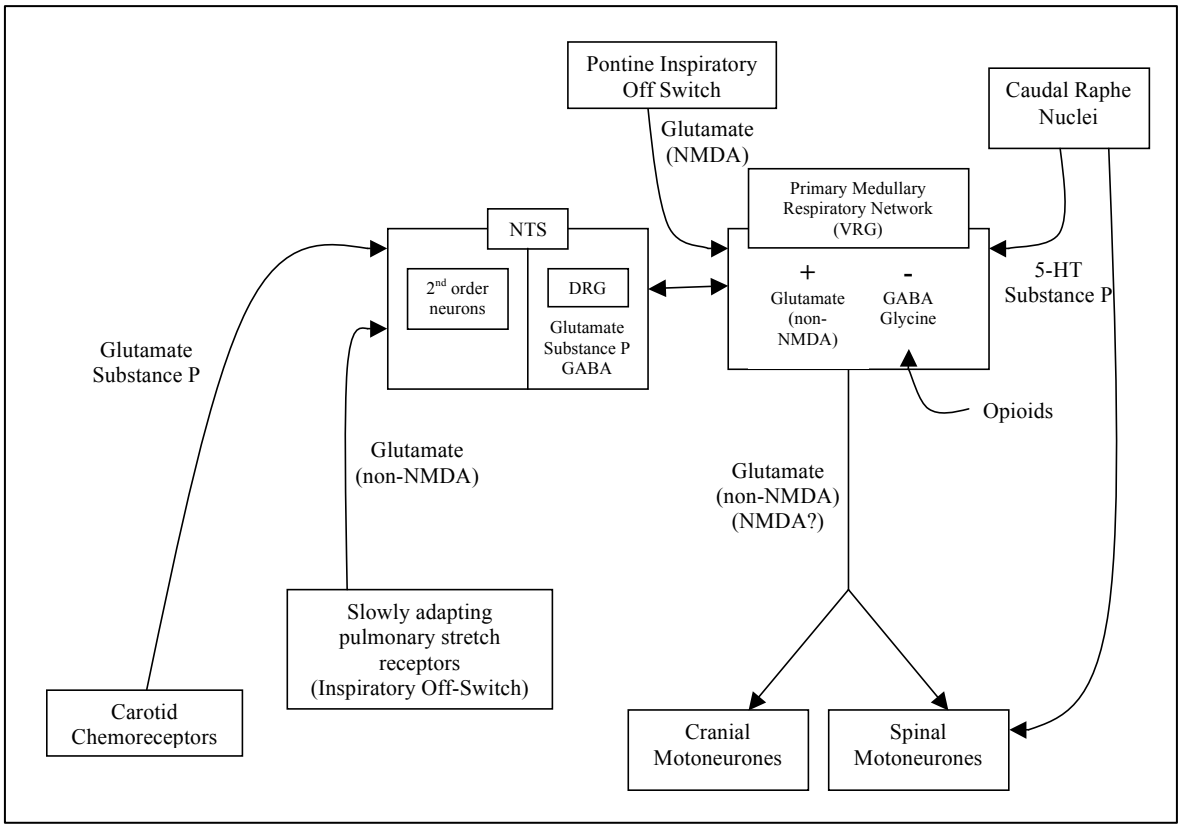


Figure 3. Schematic diagram of neurotransmitters and other neuroactive agents involved in the central control of respiration. Respiratory neurons are concentrated in two distinct medullary regions: the dorsal respiratory group (DRG) in the nucleus tractus solitarius (NTS) and the ventral respiratory group (VRG) in the ventrolateral medulla. Glutamate, gamma-aminobutyric acid (GABA) and glycine are the principal neurotransmitters involved in generating and transmitting respiratory rhythm. Glutamate may engage in N-methyl-D-aspartate (NMDA) receptors, but acts primarily at non-NMDA (e.g. AMPA) receptors. Serotonin (5-HT) also has been implicated in regulating respiratory rhythm, possibly via serotonergic projections originating in the raphe nuclei.
Figure redrawn from: Bonham AC (1995) Neurotransmitters in the CNS control of breathing. *Respiration Physiology* 101:219-230.

This neuronal control of respiration is regulated by chemical control conveyed via two sets of chemoreceptors, peripherally and centrally located. The peripheral chemoreceptors, namely the carotid and aortic bodies, are located at the bifurcation of the carotid arteries and near the arch of the aorta, respectively (Ganong 2005). These chemoreceptors are most sensitive to changes in arterial oxygen and because of their relative anatomical locations, the carotid bodies have been studied more extensively than the aortic bodies (Dahan et al. 2007; Ganong 2005). A small decrease in oxygen tension depolarizes the glomus cells of the carotid bodies by inhibiting potassium outflow through oxygen-sensitive potassium channels (Haji et al. 2000). Calcium channels are then activated, which triggers the release of neurotransmitters, such as acetylcholine (ACh), with consequent excitation of the afferent nerve endings (Haji et al. 2000; Ganong 2005). These signals are transmitted to relay neurons in the nucleus tractus solitarius (NTS) by glutamate through AMPA receptors (Haji et al. 2000).

Although the carotid and aortic bodies are most sensitive to changes in arterial oxygenation, recent evidence suggests these peripheral chemoreceptors also play a role in the overall response to hypercapnia and acidosis, although the exact mechanism of action is not well understood (Forster et al. 2008). Overall, carbon dioxide provides the major stimulus to breathe and the carbon dioxide-evoked respiratory response is mediated predominantly via the central (medullary) chemoreceptors, located within the lower brainstem, particularly in the retroptrapezoid nucleus and midline raphe nuclei (Dahan et al. 2010; Spyer & Gourine 2009). These central chemoreceptors monitor the hydrogen ion concentration of cerebrospinal fluid, including the brain interstitial fluid (Ganong 2005). Carbon dioxide that enters the brain is quickly hydrated by the catalyst carbonic anhydrase, such that bicarbonate and hydrogen ions are produced from the reaction between carbon dioxide and water. The local hydrogen ion concentration in the brain interstitial fluid therefore, parallels the arterial partial pressure of carbon dioxide (Ganong 2005).

Apart from the inputs from central and peripheral chemoreceptors, inputs from areas within the pons and cortex also modulate respiratory rhythm and breathing patterns (Dahan et al. 2010; Pattinson 2008). These sensory afferent inputs enter the medulla at the nucleus tractus solitaries where they modulate the central neuronal network responsible for respiratory rhythm generation (Smith et al. 2013).

1.5.2 Opioids and respiration

The control of respiration is a complex, integrated process, with many factors contributing to the maintenance of sufficient arterial oxygen to meet metabolic demands. With opioid-induced respiratory depression, the complexity of respiratory control is coupled with the intricate science of opioid pharmacology. Despite years of research, opioid-induced respiratory depression remains a primary concern related to the use of opioids (Dahan et al. 2010), which demonstrates the difficulty in untangling the functional relationship between opioids and respiration.

The administration of exogenous opioids is thought to affect respiration both centrally and peripherally, thereby influencing all aspects of breathing. Centrally, and most significantly, opioids have a direct inhibitory effect on the respiratory centre primarily through activation of mu-opioid receptors (Dahan et al. 2001; May et al. 1989). Mu-opioid receptors have been identified as the primary targets for the opioid-induced depression of respiratory neurons via a series of gene knock-out studies. Mice lacking mu-opioid receptors did not display morphine-induced respiratory depression (Dahan et al. 2001). As mu-opioid receptors are abundantly expressed within the brainstem respiratory centres, particularly the pre-Bötzinger complex (Montandon et al. 2011; Pattinson 2008), the most opioid-sensitive aspect of respiration is inspiratory rhythm generation (Pattinson 2008). Importantly, pre-inspiratory (expiratory) neurons in the retrotrapezoid nucleus are unaffected by opioids (Takeda et al. 2001). Consequently, opioids increase the duration of expiration, thereby decreasing respiratory frequency (Ferguson & Drummond 2006). The pre-Bötzinger complex can fully mediate the opioid-induced depression of respiratory rate because this rate depression was prevented when naloxone (an opioid antagonist) was administered at the pre-Bötzinger complex only in the presence of systemic mu-opioid agonist administration (Montandon et al. 2011).

However, opioid-induced respiratory compromise is not limited to the depression of central respiratory neurons. Together with a depression of respiratory rate, mu-opioid activation also suppresses the activity of the hypoglossal (XII) motor neurons, which innervate tongue muscles and are vital for maintaining upper-airway patency during inspiration (Hajiha et al. 2009). More peripherally, opioids induce chest and abdominal wall rigidity, which may contribute to a decreased tidal volume, and opioids blunt

respiratory responsiveness to carbon dioxide and hypoxia thus disturbing the mechanism that regulates ventilation and oxygen homeostasis (Lalley 2008; Magosso et al. 2004). Systemic administration of opioids, therefore, causes a central depression of ventilation, together with peripheral alterations that ultimately lead to hypercapnia, hypoxaemia and acidosis (Haji et al. 2000; Pattinson 2008; Shook et al. 1990).

The opioid-induced respiratory alterations are coupled with the opioid-induced cardiovascular effects, which may exacerbate the effects of poor ventilation by negatively affecting oxygen delivery. In goats, the alveolar-arterial oxygen partial pressure gradient ($P(A\text{-}a)O_2$) increased significantly within two minutes of etorphine administration, highlighting etorphine's negative effect on oxygen diffusion from the alveoli to the arterial blood (Meyer et al. 2015). The major cause for the increased $P(A\text{-}a)O_2$ following etorphine administration was attributed to pulmonary hypertension, which occurred primarily as a result of increased pulmonary vascular resistance (Meyer et al. 2015). The exact mechanism behind the etorphine-induced increase in pulmonary vascular resistance is not clearly understood, but it may result from an opioid-induced activation of the sympathetic nervous system (Haigh 1990; Heard et al. 1990; Heard et al. 1996; Roquebert & Delgoulet 1988) or from the indirect effects of opioid-induced hypoxaemia, which leads to vasoconstriction in the pulmonary vasculature (Lumb 2005b). In immobilized wildlife, these deleterious cardiorespiratory effects elicited by opioid administration are compounded by the heightened sympathetic response directly related to the capture event.

1.5.3 Opioid-induced respiratory depression in herbivores

Most wildlife species immobilized with opioid combinations, in free-ranging and captive conditions, show markedly decreased arterial oxygen levels indicating severe hypoxaemia (Fahlman 2008). However, there is considerable variation between species in the pharmacodynamic responses to opioid administration. Some species, including giraffe, impala and white rhinoceros, are more prone to developing life-threatening respiratory depression than are others (Swan 1993).

The white rhinoceros in particular, is extremely sensitive to etorphine-induced respiratory depression (Burroughs et al. 2012a; Heard et al. 1992; Rogers 1993). All rhinoceros experience some degree of hypoxaemia, often with P_aO_2 values well below 60 mmHg,

when under etorphine immobilization (Burroughs et al. 2012a; Bush et al. 2004; Heard et al. 1992). Although vast improvements have been made to rhinoceros immobilization protocols, it is no longer sufficient to judge success purely on decreased mortality rates during the capture event. With improved physiological monitoring capabilities, hypoxaemia has been identified as one of the most significant and potentially life-threatening side-effects of potent opioid anaesthetic agents in rhinoceros (Heard et al. 1992; Kock et al. 1995; Rodgers 1993).

This respiratory depression is dose dependent (Portas 2004) and therefore rhinoceros immobilized in the field, rather than in a captive environment are generally more vulnerable to hypoxaemia and the consequent complications. During field immobilizations the stress and exertion encountered during pursuit and capture are thought to create a physiological cascade with hyperthermia, anaerobic glycolysis, metabolic acidosis, reduced tissue perfusion and hypoxia (Spraker 1993), resulting in necrosis of cardiac and skeletal muscles (Williams & Thorne 1996). Therefore, it is preferable to use the highest dose possible to immobilize the animal as quickly as possible, as extended times and distances to recumbency have been shown to result in greater adverse physiological responses (Cheney & Hattingh 1987). A consequence of higher etorphine doses, however, is exacerbated respiratory depression.

1.5.4 Consequences of opioid-induced respiratory depression for animals

As described above, hypoxaemia has been identified as one of the most significant and life-threatening side-effects of opioid-induced immobilization in wildlife. Low levels of oxygen in arterial blood will result in inadequate oxygen delivery leading to tissue hypoxia. As the body has a very limited reservoir of oxygen, lack of blood oxygen for more than a few minutes can cause irreversible damage to the most sensitive organs, including the brain and heart (Feldman & Del Negro 2006). When tissues become hypoxic, anaerobic metabolism occurs with the resultant production of hydrogen and lactate ions, which are released into the circulation. As the blood-brain barrier is relatively impermeable to charged ions, the hydrogen and lactate ions are retained within the neurons of the hypoxic brain (Lumb 2005a). The hypoxic neurons undergo abnormalities in channel function, which destroys ionic gradients and ultimately results in cell death (Lumb 2005a). The heart is another organ that is very susceptible to hypoxic damage,

especially during anaesthesia. Myocytes are extremely sensitive to a decrease in oxygen supply and can stop contracting within seconds from the onset of oxygen deprivation (Ostadal et al. 1999). Moreover, general hypoxaemia causes activation of the sympathetic nervous system and release of catecholamines. Consequently, heart rate and myocardial contractility will be increased, thus increasing the cardiac oxygen demand (Wilson & Benumof 2005). Thus an increase in oxygen demand coupled with a decrease in oxygen supply will promote cardiac irritability and arrhythmias. In the presence of severe hypoxaemia, local depressant effects dominate and blood pressure falls rapidly, with ensuing shock resulting in cardiac fibrillation or arrest (Wilson & Benumof 2005).

If the animal survives these acute hypoxic challenges during immobilization, subclinical damage to vital organs may lead to the slow deterioration of normal function. Long-term monitoring post immobilization is not often pursued, thus we are not fully aware of potential adverse consequences related to severe acute physiological disturbances that occur during immobilization. In Canada, researchers found that immobilization negatively affected both ranging behaviour and body condition of grizzly and black bears (Cattet et al. 2008). Immobilization and handling of polar bears has also been suggested to adversely affect female condition and cub survival rates (Dyck et al. 2007). Indeed, female animals and their offspring are likely to be more prone to immobilization-induced disturbances than are males. One study found that moose cows immobilized with opioid combinations during the last three months of pregnancy gave birth to calves with reduced postnatal survivorship (Larsen & Gauthier 1989). Female moose also changed movement patterns for 4.5 days post immobilization (Neumann et al. 2011). In the critically endangered black rhinoceros, repeated immobilizations have reportedly had a negative effect on the fertility of females, a potentially serious consequence especially when the end goal is population expansion (Alibhai et al. 2001). Admittedly, a multitude of factors may contribute to the chronic negative consequences of immobilization. However, to limit the risk of deleterious immobilization-induced side-effects, it would be prudent to minimize hypoxic-induced cellular damage.

With opioid-induced ventilatory insufficiency, an increase in arterial carbon dioxide accompanies the decrease in arterial oxygen. Increased arterial carbon dioxide causes the blood pH to decrease, which decreases haemoglobin affinity for oxygen (Bohr effect). The resulting release of oxygen is beneficial for hypoxic tissues, but not beneficial to

uploading oxygen onto haemoglobin in the lungs. Moreover, severe hypercapnia and acidosis causes an increase in plasma potassium, which decreases myocardial contractility and predisposes the animal to arrhythmias and ultimately heart failure (Johnson & De Morais 2012; Meltzer & Kock 2012). Hypercapnia can also depress the central nervous system, including the respiratory centre, and can eventually lead to unconsciousness and death (Ganong 2005).

In field immobilizations, the deleterious effects of potent opioid administration are accompanied by the physiological changes that occur as a result of over-exertion and high sympathetic activity induced by the stress of capture (Meltzer & Kock 2012). In the presence of poor oxygen supply to the muscles, stress and over-exertion can result in the condition known as capture myopathy (Meltzer & Kock 2012). The damage caused by capture myopathy is seen in both the myocardium and skeletal muscles and can result in acute death, or chronic irreversible pathology that ultimately causes the demise of the animal.

1.6 Treatments for opioid-induced hypoxaemia

Because opioids are used widely for their analgesic effects, researchers have investigated ways to prevent or treat opioid-induced respiratory compromise. Although there have been significant improvements in opioid administration protocols in an attempt to harness their beneficial effects while minimising their deleterious side-effects, opioid deaths directly related to respiratory depression still occur.

1.6.1 Partial and full opioid antagonists

In human medicine, naloxone is currently the treatment of choice for opioid-induced respiratory depression (Van der Schier & Roozekrans 2015). Naloxone, first synthesized in 1960, is a non-competitive opioid antagonist at the mu-, delta-, and kappa-opioid receptors, but displays greatest affinity for the mu-opioid receptor (Santiago & Edelman 1985). The administration of naloxone, therefore, will reverse all pharmacological effects of opioids, including analgesia, sedation, catatonia and respiratory depression, in a dose-dependent fashion. Naloxone's relatively short duration of action, low potency and cost preclude its general use as an opioid antagonist in wildlife (Swan 1993). Thus, naltrexone,

another full-opioid antagonist with a longer duration of action, and greater potency than naloxone, is the preferred antagonist when a full recovery from opioid-induced immobilization in wildlife is required (Burroughs et al. 2012b).

Before naloxone was made available, nalorphine was the agent used in humans to reverse severe opioid-induced respiratory depression. Nalorphine restored respiratory rate and tidal volume, while also maintaining some analgesia (Van der Schier & Roozekrans 2015). Nalorphine is classified as a mixed opioid agonist-antagonist as it has antagonistic activity at mu-receptors, and acts as a partial agonist at kappa-receptors (Shook et al. 1990). With regard to binding affinity, nalorphine interacts with mu-, delta- and kappa-receptors, in that order, implying that it is more potent as a mu-antagonist than as a kappa-agonist (Shook et al. 1990). This mixed agonist-antagonist property of nalorphine prompted wildlife veterinarians to use this drug in an attempt to improve respiration, while maintaining immobilization in etorphine-immobilized rhinoceros (Kock et al. 1995; Burroughs et al. 2012a). One study suggested that a standard protocol for white rhinoceros immobilization should involve the administration of 10-20 mg intravenous nalorphine as soon as possible after recumbency, which would counteract respiratory depression and hypoxaemia, resulting in an increased oxygen saturation of haemoglobin (SpO_2) within 5 minutes (Kock et al. 1995). However, another study reported that nalorphine administered to free-ranging rhinoceroses after they became recumbent resulted in only a minor improvement of 7 mmHg in the partial pressure of arterial oxygen (Bush et al. 2004).

A similar suggested protocol is to give 1 mg diprenorphine together with 10 mg nalorphine intravenously immediately to all immobilized white rhinoceroses (Burroughs et al. 2012a). Diprenorphine is another mixed opioid agonist-antagonist with relatively potent antagonistic activities, thus it also is commonly used as an antidote for etorphine in species other than white rhinoceros (Burroughs et al. 2012b). Unfortunately, nalorphine is no longer readily available as it causes dysphoric and psychotomimetic side-effects in humans (Gutstein & Akil 2001). Therefore, wildlife veterinarians have turned to alternative opioid agonist-antagonists in an attempt to treat opioid-induced respiratory depression in the most sensitive species such as white rhinoceros.

Butorphanol and nalbuphine are two alternative opioid agonist-antagonists with similar properties to nalorphine, in that they have predominantly mu-antagonistic and kappa-

agonistic activities (Hoskin & Hanks 1991). Like nalorphine, nalbuphine and butorphanol should therefore produce analgesia, catalepsy and sedation with little or no respiratory depression (Bowdle 1998). Butorphanol is a synthetic, weak mu-receptor antagonist (or a partial mu-agonist), a pure kappa-agonist and a delta-agonist with high receptor affinity (Commiskey et al. 2005; Hoskins & Hanks 1991). Although butorphanol is said to interact with all its receptors at about the same concentration (Shook et al. 1990), some suggest that the pharmacological responses resulting from butorphanol activity at mu-receptors frequently overshadow those of the kappa-receptors (Commiskey et al. 2005). In humans, intravenous butorphanol reversed the respiratory depressant effects of fentanyl without affecting analgesia in post-surgery patients (Bowdle et al. 1987). Butorphanol therefore appears to be a promising agent for the reversal of etorphine-induced respiratory depression while still maintaining adequate sedation.

Indeed, in wildlife immobilization protocols many veterinarians use butorphanol to reverse etorphine-induced respiratory compromise (Burroughs et al. 2012b). However, recent studies in rhinoceros indicate that butorphanol is not as effective a mu-antagonist as previously thought and that butorphanol's beneficial respiratory effects are related more to a decrease in immobilization level rather than to antagonism of mu-opioid induced hypoventilation (Miller et al. 2013; Wenger et al. 2007). This conclusion was based on the persistence of hypoxaemia in rhinoceros given butorphanol during immobilizations.

Evidence from human and rodent studies indicates that nalbuphine, another mixed opioid agonist-antagonist that is currently not used in veterinary medicine, may be a better mu-opioid antagonist than is butorphanol or nalorphine (Bowdle et al. 1987; Pallasch & Gill 1985; Schmidt et al. 1985; Zucker et al. 1987). Nalbuphine is between 0.8 and 0.9 the potency of morphine when the total analgesic effect is considered (Beaver & Feise 1978) and in humans it has less psychotomimetic effects than does nalorphine (Romagnoli & Keats 1980).

Responses to mixed opioid agonist-antagonists may differ across species. For example, in non-human primates, butorphanol has been characterized as a low-efficacy mu-receptor agonist, rather than as a mu-antagonist (Butelman et al. 1995; Vivian et al. 1999), and the respiratory responses to butorphanol differ between rats and dogs (Shook et al. 1990). Therefore, in order to accurately identify the positive respiratory effects of mixed opioid

agonist-antagonists, and to determine which mixed drugs will elicit the greatest positive response in immobilized wildlife, the drugs need to be systematically evaluated in appropriate herbivore models.

Specific mixed opioid agonist-antagonists may indeed be useful in some immobilization protocols. However, with the increased understanding of opioids and their receptors, it is becoming evident that it may be impossible to completely separate the beneficial effects of opioids, such as analgesia, sedation and catatonia, from respiratory depression (Dahan 2007). Also, given the differences in responses to opioid ligands within and between species, it may be impossible to find an opioid agonist-antagonist that uniformly targets opioid receptors in a beneficial manner in respiratory-compromised individuals of all species. Therefore we should seek alternative pharmacological interventions that reverse opioid-induced respiratory depression without affecting the intended use of the opioid agonists, like analgesia or the level of immobilization.

1.6.2 Analeptics and chemoreceptor stimulants

Doxapram, an analeptic agent, often is used to stimulate breathing in anaesthetised animals. Analeptic drugs cause widespread central nervous system stimulation, with resultant increased ventilation (Bowdle 1988). Indeed, in human medicine, doxapram has been used to arouse patients in the post-anaesthetic setting as well as for emergency treatment of drug-induced central nervous system depression (Yost 2006). Apart from its central nervous system stimulation, doxapram also directly stimulates nerve endings on the carotid bodies through inhibition of potassium channels, which leads to a local release of neurotransmitters, much like the effects of hypoxia (Golder et al. 2013; Yost 2006). Thus, the stimulatory effect of doxapram on carotid chemoreceptors is independent of the arterial partial pressures of oxygen and carbon dioxide (Golder et al. 2013).

In early studies in postoperative human patients, doxapram caused an immediate moderate to intense increase in minute volume but the effect dissipated within 5 to 8 minutes (Stephen & Talton 1964). Similarly, doxapram has been used to stimulate respiration in immobilized wildlife and while it is advantageous for its quick onset of action, the disadvantage of this drug is that the beneficial effects are short-lived (Burroughs et al. 2012b; Kock et al. 1995). Moreover, doxapram may stimulate ventilation beyond baseline

levels, thereby causing hypocapnia and a respiratory alkalosis (Bleul et al. 2010; Yost 2006). A potential negative consequence of hypocapnia is cerebral vasoconstriction with subsequent cerebral hypoxia (Laffey & Kavanagh 2002).

Another negative effect of doxapram administration is the proposed catecholamine release (Yost 2006), which results in increased cardiac workload associated with hypertension and increased cardiac output (Van der Schrier et al. 2014). This increased cardiac workload will lead to greater cardiac oxygen consumption (Bleul et al. 2010). The stimulated cardiac work together with a hyperventilatory response will result in oxygen consumption and carbon dioxide production increasing in parallel with the increase in minute ventilation (Plumb 2002). Thus, although doxapram will improve the rate and depth of breathing, it may have little effect on the oxygen concentration of arterial blood when used alone in immobilized wildlife (Portas 2004). Indeed, because of its side-effect profile including hypertension, anxiogenesis, dyspnoea and negative effects on cerebral oxygenation, doxapram these days is seldom used in adult humans (Golder et al. 2013; Yost 2006).

Although doxapram has disadvantages to its use, a selective carotid body stimulant with limited sympathomimetic effects may provide a suitable treatment option for opioid-induced respiratory depression (Golder et al. 2013). Almitrine has a similar mode of action to that of doxapram, but with minimal cardiovascular effects (Golder et al. 2013). Like doxapram, almitrine causes tidal volume and respiratory rate to increase by stimulating peripheral chemoreceptors in a manner similar to that of hypoxic stimulation (Golder et al. 2013; Laubie & Schmitt 1980). Almitrine would therefore appear to be a useful respiratory stimulant in the face of opioid-induced respiratory depression but its use also has been limited as a result of potential peripheral neuropathy caused by its metabolites (Golder et al. 2013; Howard 1989).

GAL-021 is a newer potassium channel blocker that acts at the carotid bodies and mimics the effects of hypoxia (Roozekrans et al. 2015). It is a derivative of almitrine but is devoid of the harmful metabolites and has been shown to increase respiratory rate and tidal volume without affecting analgesia and sedation in alfenatil sedated human patients (Roozekrans et al. 2015). However, GAL-021 has a short duration of action if a continuous infusion is not maintained (Roozekrans et al. 2015). Also, the effect of GAL-

021 is less pronounced in severe opioid-induced respiratory depression (Van der Schrier & Roozekrans 2015), a situation that commonly occurs in animals immobilized with potent opioid combinations. In general, it is now thought that the efficacy of drugs acting via carotid body stimulation may be limited in their ability to reverse severe opioid induced respiratory depression (Roozekrans et al. 2015). Thus, although doxapram and GAL-021 may have some beneficial effects in patients experiencing opioid-induced respiratory depression, they are not ideal agents for use in etorphine-immobilized herbivores due to their short duration of action, generalized stimulatory effects and potential limited efficacy.

1.6.3 Drugs that increase cyclic adenosine monophosphate (cAMP)

An alternative means to specifically treat opioid-induced respiratory depression is to use agents that activate pathways to positively affect the production of cAMP in the central respiratory network (Lalley 2005). Not only is the cAMP-protein kinase A pathway in neurons essential for the generation of respiratory rhythm (Ballanyi et al. 1997), it also is the pathway that is inhibited by mu-opioid receptor activation (Haji et al. 2000). cAMP can be increased either by inhibiting the enzyme phosphodiesterase, which catabolizes cAMP, or by increasing the activity of adenylyl cyclase, the enzyme that converts ATP to cAMP.

Methylxanthines, such as caffeine, aminophylline and theophylline play a role in the cAMP-mediated modulation of respiratory neurons (Wilken et al. 2000). There is some controversy regarding the exact underlying mechanism of methylxanthines, but it is generally accepted that the administration of methylxanthines results in an increase in cAMP (Wilken et al. 2000). At high concentrations, methylxanthines block the catabolic enzyme, phosphodiesterase 4 (PDE4), which results in elevated cAMP and stimulation of phosphokinase A (Fredholm et al. 1999). Peripherally, phosphodiesterase inhibition also is thought to contribute to bronchial muscle relaxation and an increase in the activity of inspiratory muscles (Nishii et al. 2008; Yokoba et al. 2015). However, at concentrations applicable to *in vivo* studies, methylxanthines act primarily by blocking adenosine A_1 receptors (Fredholm et al. 1999; Wilken et al. 2000). Adenosine receptors are persistently active in the respiratory network and play a role in inhibiting adenylyl cyclase (Schmidt et

al. 1995). Thus methylxanthines are thought to disinhibit adenylyl cyclase through their action on adenosine A_1 receptors, which results in a rise in cAMP (Wilken et al. 2000).

Although the methylxanthine, caffeine, was one of the first agents to treat opium and morphine poisoning, studies with caffeine have found only modest respiratory stimulatory effects (Van der Schrier & Roozekrans 2015). Thus, caffeine is no longer considered capable of reversing opioid-induced respiratory depression (Van der Schrier & Roozekrans 2015). However, in human medicine, methylxanthines still have a role to play in non-opioid related respiratory conditions such as in asthmatic and neonatal patients (Lam & Newhouse 1990; Picone et al. 2014).

Methylxanthines may not be ideal for reversing opioid-induced respiratory depression, but other agents that increase cAMP more specifically in respiratory neurons may provide relevant treatment options for improving respiration in opioid-immobilized herbivores (Ballanyi et al. 1997). Also, with an increased understanding of the neurotransmitters that directly modulate respiratory rhythm, it may be possible to manipulate specific neuronal transmitter systems within the pre-Bötzinger complex to restore respiratory drive that has been dampened by opioid administration.

Serotonin and glutamate are two such neurotransmitters that are implicated in a wide variety of physiological processes through the activation of various receptor subtypes often leading to activation of adenylyl cyclase with subsequent increases in cAMP (Dutschmann et al. 2009; Richter et al. 2003). Serotonin modulates respiratory rhythm generation through its actions on $5HT_{1A}$, $5HT_4$ and $5HT_7$ serotonin receptors (Mironov & Richter 2000; Ramiraz et al. 1997; Richter et al. 2003). 5-HT_4 and 5-HT_7 receptors are linked to G_s proteins, leading to activation of adenylyl cyclase with subsequent increases in cAMP (Richter et al. 2003). Thus, 5-HT_4 and mu-opioid receptors, which are co-expressed on respiratory neurons in the pre-Bötzinger complex, display opposing actions (Dutschmann et al. 2009). Mu-opioid receptor activation decreases intracellular cAMP, causing reduced excitability of neurons with resultant decreased inspiratory drive (Manzke et al. 2003), while stimulation of 5-HT_4 receptors increases intracellular cAMP leading to increased respiratory drive and thus restoration of normal breathing (Manzke et al. 2003). As 5-HT_4 receptors are not expressed on pain neurons, activation of 5-HT_4 receptors will not affect the opioid-induced analgesia (Manzke et al. 2003). A study using the 5-HT_4

agonist BIMU8, confirmed that this serotonergic agonist can reverse fentanyl-induced respiratory depression and apnoea, without affecting antinociception in rats (Manzke et al. 2003). More relevant to herbivores is the finding that zacopride, another 5-HT_4 receptor agonist, reversed etorphine-induced respiratory depression in goats without affecting the catatonic immobilization (Meyer et al. 2006). However, contrary to these animal trials, the orally administered 5-HT_4 receptor agonist, mosapride, failed to treat morphine-induced respiratory depression in humans (Lötsch et al. 2005). This negative result with the clinically available 5-HT_4 agonist could have been related to low brain concentrations after oral administration or insufficient potency of the agonist (Lötsch et al. 2005). Unfortunately, more potent, or better brain-penetrating 5-HT_4 agonists are currently unavailable for clinical use (Dahan 2007; Lötsch et al. 2005).

Activation of 5-HT_{1A} receptors also has been shown to restore breathing after opioid-induced depression as well as to maintain, or even enhance opioid analgesia (Guenther et al. 2009). The exact mechanism of respiratory stimulation by 5-HT_{1A} receptors is not well understood, but 5-HT_{1A} receptors are densely expressed within key brainstem regions that control cardiorespiratory function (Dutschmann et al. 2009). The exogenous serotonin ligands 8-OH-DPAT, an agonist at the 5-HT_{1A} and 5-HT_7 receptors, and buspirone, a partial agonist at the 5-HT_{1A} receptor reversed opioid-induced depression of respiratory neurons in anaesthetised rats (Manzke et al. 2003; Sahibzada et al. 2000).

Apart from the direct stimulatory actions of serotonergic agonists within the pre-Bötzinger complex, the cardiovascular effects of serotonergic agonists also has been proposed as an important factor in the improvement of oxygenation following the administration of serotonergic agonists in the presence of potent opioids. In opioid-immobilized goats that received serotonergic agonists, oxygen partial pressures improved without a change in ventilation (Meyer et al. 2006). It was proposed that the improvement in oxygenation occurred as a direct action of the agonists on pulmonary vessel diameter, thereby improving lung perfusion (Meyer et al. 2006). Also, in etorphine-immobilized impala, both metoclopromide (a 5-HT_4 agonist) and buspirone (a 5-HT_{1A} agonist) improved the respiratory function without reversing the immobilization (Meyer et al. 2010). These two drugs decreased the differences between alveolar and arterial partial pressures of oxygen, presumably by improving ventilation-perfusion ratios (Meyer et al. 2010).

Although metoclopromide and buspirone improved blood oxygenation in impala with opioid-induced respiratory depression, the hypoxaemia was not completely resolved (Meyer et al. 2010) and, in humans, buspirone failed to counteract opioid-induced ventilatory depression (Oertel et al. 2007). Buspirone, the only commercially available 5-HT_{1A} receptor agonist for use in humans, is a partial 5-HT_{1A} receptor agonist and available only for oral intake (Guenther et al. 2012). Therefore, until more potent and specific serotonergic agonists become available, their clinical use for opioid-induced respiratory depression remains limited.

1.6.4 Glutamate receptor modulators

Glutamate receptors mediate a major component of excitatory synaptic transmission (Ge & Feldman 1998). Within the respiratory centre, activation of non-NMDA glutamate receptors is necessary for respiratory rhythm generation (Greer et al. 1991; Smith et al. 1991). Specifically, it has been demonstrated in laboratory models that glutamate, acting via α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, is a critical component for the generation and modulation of respiratory rhythm within the pre-Bötzinger complex (Funk et al. 1993; Ge & Feldman 1998; Greer et al. 1991; Pace et al. 2007). Blocking AMPA receptors in the pre-Bötzinger complex completely abolished respiratory motor discharge and rhythmic drive in respiratory neurons, while local amplification of AMPA to the pre-Bötzinger complex increased respiratory frequency (Ge & Feldman 1998).

AMPA receptors are tetramers composed of various combinations of 4 subunits (GluR1-4) (Lynch 2006). Different regions of the brain have AMPA receptors composed of different combinations of subunits (Gold et al. 1997; Martin et al. 1993) and AMPA receptors can be positively modulated by phosphorylation via a number of small molecules (Ge & Feldman 1998). The group of modulators collectively called ampakines has proved to be the most interesting to date with regards to respiratory rhythm generation (Dahan et al. 2010). AMPA receptor modulators do not interact with the receptor site directly as agonists (or antagonists), but bind to an allosteric site within the receptor (Boom et al. 2012), thereby increasing the duration of glutamate-induced AMPA receptor-gated inward currents (Arai et al. 2004; Greer et al. 1991). Thus ampakines positively alter channel kinetics at AMPA receptors and it is the enhancement of glutamate binding affinity and

prolongation of channel open time that is the important parameter in the action of ampakines in relation to controlling activity of respiratory networks (Ren et al. 2006).

Ampakines act directly within the pre-Bötzinger complex to increase respiratory frequency, but premotor and motor neuron pools also are likely sites of ampakine action (Ren et al. 2006). Indeed, given the ubiquitous distribution of AMPA receptors and glutamatergic transmission within the central nervous system, it may be expected that stimulation would result in widespread activation of neuronal circuits, resulting in undesirable side-effects (Ren et al. 2006). However, many ampakines have clear receptor subunit preferences and could therefore act in a regionally selective fashion (Lynch 2006). Moreover, ampakines have been shown to preferentially enhance the response in regions of the brain with subnormal activity (Lynch 2006). It is therefore possible that ampakines display regional and network selectivity, and AMPA modulation may occur within specific malfunctioning areas (Dahan et al. 2010; Ren et al. 2006).

To date, a number of ampakines have been developed and tested in experimental settings for their potential to reverse opioid-induced respiratory depression. The most extensively studied ampakine, CX717, readily crosses the blood-brain barrier, is metabolically stable and does not produce significant unwanted side-effects (Ren et al. 2009). CX717 has been shown to block as well as reverse respiratory depression induced by the mu-opioid agonist fentanyl, without suppressing analgesia in rats (Ren et al. 2009). Trials with CX717 have been extended to humans where pain tolerance to heat and electrical stimuli did not decrease in patients with alfentanil-induced respiratory depression that had been treated with CX717 (Oertel et al. 2010). One caveat with the use of CX717 is that sedation was reported to increase in the presence of CX717 (Oertel et al. 2010). While increased sedation may be an undesirable side-effect in humans, for wildlife immobilization, improved respiration while maintaining or even improving sedation may be an advantage to the use of ampakines during chemical immobilization.

Another ampakine, CX546, also acted as a powerful stimulant of respiratory frequency and tidal volume without affecting analgesia in rats affected by mu-opioid receptor depression of respiratory neurons (Ren et al. 2006). Importantly, in adult rats ampakines enhanced respiratory frequency only if respiration was initially suppressed by opioid administration (Ren et al. 2006). Similarly, CX717 did not elevate respiratory or

cardiovascular parameters above baseline conditions in human studies (Wesensten et al. 2007). The mechanism underlying the preferential potentiation of suppressed networks by ampakines is not fully understood (Lynch 2004, Lynch 2006), but this property is desirable from a therapeutic perspective as it will reduce the likelihood of side-effects (Lorier et al. 2010).

To date, the majority of ampakines tested lack solubility and need to be dissolved in either dimethyl sulfoxide (DMSO) or 2-hydroxypropyl-beta-cyclodextrin (HPCD). DMSO cannot be combined with potent opioids, such as etorphine, as DMSO enhances the absorption of etorphine through the skin, thus significantly increasing the risk of fatal accidental administration in humans. A newer ampakine, CX1942, is water soluble and therefore may be more appropriate for wildlife use (Greer, personal communication), although reports on its efficacy are not yet available.

1.6.5 Microglial inhibitors

Apart from manipulating the neuronal effects of opioids, there may be scope to produce opioids with reduced respiratory depressant effects by manipulating the role of immune cells in opioid-related mechanisms (Boom et al. 2012). It has recently been elucidated that opioids may interact with microglia via non-opioid receptors, for example the toll-like receptor 4 (TLR4) protein (Boom et al. 2012). It is now clear that glial cells and TLR4 receptors are implicated in the overall analgesic effects of opioids, therefore, they also may be involved in other opioid-induced effects such as respiratory depression (Boom et al. 2012). In brain slices, specific blockade of the glial Krebs cycle has been shown to suppress rhythmic respiratory burst activity (Hülsmann et al. 2000) and an *in vivo* study has described the role of glial cells and astrocytes in influencing central carbon dioxide chemosensitivity and ventilation (Erlichman & Leiter 2010). Minocycline is a semi-synthetic, second-generation tetracycline antibiotic, first introduced in 1972, and is known to possess anti-inflammatory and neuroprotective effects unrelated to its antibacterial activitiy (Kim & Suh 2009). Via the inhibition of microglia, minocycline also influences respiratory parameters. In the rat model, the presence of minocycline suppressed the morphine-induced changes in tidal volume, minute ventilation, inspiratory force, expiratory force and blood oxygen saturations, although it did not block morphine-induced changes in respiratory rate (Hutchinson et al. 2008). In addition, minocycline enhanced the

analgesic effects of morphine (Hutchinson et al. 2008). Hence, minocycline suggests an interesting possibility for the development of inhibitors of glial activation for reversal or prevention of opioid-induced respiratory depression that may simultaneously enhance opioid-induced analgesia.

1.6.6 Increased inspired oxygen

Another method of improving arterial oxygenation during chemical immobilization is to increase the fractional inspired concentration of oxygen (FiO_2), thus creating a greater oxygen diffusion gradient between the alveoli and the pulmonary capillaries. Oxygen supplementation is standard protocol in veterinary hospitals where the majority of invasive procedures are carried out under gaseous anaesthesia. However, primarily for practical reasons, oxygen supplementation is not widely considered for immobilized wildlife. Recently, studies have shown that oxygen supplementation significantly improves arterial oxygenation and indeed it is recommended for several large wildlife species (Bush et al. 2004; Fahlman 2008; Fahlman et al. 2012 Fahlman 2014; Lian et al. 2014; Mich et al. 2008; Portas 2004). Oxygen can be administered to anaesthetised animals intranasally, via face mask, endotracheal tube, tracheal catheter, or with flow-by technique (Fahlman 2014), but not all methods of oxygen delivery are practical for field purposes. Intranasal oxygen insufflation is a simple and effective method for increasing arterial oxygenation, but the FiO_2 will vary considerably within and between species according to oxygen flow rate, minute ventilation and breathing pattern (Fahlman 2014). In brown bears weighing up to 250 kg, intranasal oxygen insufflation, administered at relatively low flow rates of 0.5 to 3 L/min, markedly improved arterial oxygenation (Fahlman et al. 2014). In white tailed deer immobilized with a combination of butorphanol, azaperone and medetomidine, intranasal oxygen at a flow rate of 3 L/min significantly improved the arterial partial pressures of oxygen and oxygen saturations (Mich et al. 2008). However, even lower flow rates, closer to 1 L/min, may be adequate to treat hypoxaemia in immobilized white tailed deer (Fahlman 2014). Nasotracheal oxygen insufflation has been evaluated for the treatment of severe hypoxaemia in adult and subadult white rhinoceros (Bush et al. 2004). Oxygen was administered at a flow rate of 15-30 L/min, which significantly improved oxygenation in most of the rhinoceros, although some individuals remained hypoxaemic (Bush et al. 2004). Oxygen is a drug and therefore the response to oxygen administration should be monitored and the therapy adjusted accordingly (Fahlman 2014). Ideally,

especially in remote wildlife areas where refilling oxygen cylinders may be practically challenging, the lowest possible oxygen flow rate needed to maintain normoxia should be used (Fahlman 2014). When oxygen supplementation is discontinued, hypoxaemia quickly recurs, suggesting that continuous oxygen supplementation is necessary to avoid hypoxaemia during immobilization (Fahlman 2014; Fahlman et al. 2014).

1.7 Summary and aims

Chemical immobilization of large wild herbivores is essential for the successful implementation of many conservation management plans in southern Africa. Also, wildlife management is integral to the growth of the ecotourism industry. The advent of potent opioids, such as etorphine, not only enabled more proactive conservation efforts, but also facilitated the rapid growth of southern Africa's wildlife industry as a whole. Potent opioids fulfil many of the requirements of an ideal immobilizing agent, such as their ability to induce a rapid induction with a small volume of drug, and they are reversible with the administration of an antagonist (Haigh 1990). However a significant, and potentially life-threatening drawback to the use of potent opioids is their negative effect on respiration, which leads to hypoxaemia, hypercapnia and acidosis. Indeed, morbidity and mortality rates directly related to the capture event are still unacceptably high (Knottenbelt 1990; Kock et al. 1987; Kock et al. 1990; Kock et al. 1995; Meltzer & Kock 2012).

Over the past few decades, significant improvements have been made to immobilization protocols, especially in the most opioid-sensitive animals, such as white rhinoceros. For example, the partial opioid agonist-antagonist butorphanol is now commonly integrated into white rhinoceros immobilization protocols. However, many of the suggested improvements for chemical capture have been based on anecdotal evidence and the monitoring of fairly non-specific physiological parameters such as respiratory rate. Respiratory rate can vary widely depending on the individual and the specific circumstances around the capture event. Also, a normal respiratory rate does not necessarily equate to adequate ventilation. Some wildlife veterinarians are now using pulse oximetry to help gauge the level of oxygenation in the animal. While pulse oximetry is a better method to assess oxygenation than respiratory rate, poor performance of pulse

oximeters may occur due to movement, bright ambient light or poor peripheral perfusion (Fahlman 2008).

The gold standard for the assessment of ventilation is arterial blood gas analysis. Monitoring the partial pressure of arterial oxygen and carbon dioxide, as well as the acid-base status of the animal throughout immobilization, is a valuable means for the evaluation of physiological effects that different chemical immobilization protocols have on wild animals (Fahlman 2008). Indeed, arterial blood gas analysis is strongly advocated as an investigative approach to reliably examine the respiratory depressant effects of opioids (Whiteside et al. 2015). As arterial blood gas analyses are expensive, and often impractical in field conditions, many of the anecdotal protocols currently used in the field have not been adequately assessed to determine their true physiological value.

Therefore, although advances in our capture techniques and drug protocols have undoubtedly improved our ability to manage wild animals, I believe there is still large scope to improve the welfare of wild animals that undergo chemical immobilization. Indeed, long-term monitoring of captured animals after the immobilization event is rarely carried out, resulting in a dearth of knowledge regarding the more chronic, potentially negative implications of opioid-induced immobilization, such as decreased fertility.

Today, rhinoceros poaching is escalating at an unprecedented rate, yet again placing these iconic, prehistoric mammals in danger of extinction. More sophisticated and intensive actions are therefore needed if we are to save this species from disappearing at the hands of poachers. We cannot afford for the immobilization procedure to place any additional risk on even a single animal. It is imperative that during translocation, dehorning, identity marking and other veterinary interventions, that rhinoceros are kept as physiologically stable as possible.

Current immobilization protocols for the white rhinoceros recommend the administration of butorphanol at a ratio of 20 mg butorphanol per mg etorphine as soon as the rhinoceros is reached after it has gone down (Burroughs et al. 2012a). While butorphanol appears to improve breathing in the immobilized rhinoceros, some reports suggest that the increased respiratory rate does not necessarily correlate with a clinically significant improvement in blood and tissue oxygenation (Bush et al*.* 2004; Miller et al. 2013; Wenger et al. 2007).

Indeed, butorphanol's beneficial respiratory effects may be related more to a decrease in immobilization level than to antagonism of mu-opioid-induced hypoventilation (Miller et al. 2013).

Therefore, it would be beneficial to test nalbuphine, another available mixed opioid agonist-antagonist, for its ability to reverse etorphine-induced respiratory depression without compromising the immobilization. Nalbuphine is not commonly used in veterinary medicine, but evidence from human and rodent studies implies that it may be a more potent mu-antagonist than is butorphanol (Pallasch & Gill 1985; Schmidt et al. 1985; Zucker et al. 1987). I therefore set out to compare and evaluate the effectiveness of butorphanol and nalbuphine in reversing opioid-induced respiratory compromise. I used the domestic goat in a laboratory setting as a model for a wild ungulate so that the drug effects could be accurately assessed without the background of a severe stress response that invariably accompanies the capture of wild animals.

Oxygen supplementation is another intervention used by veterinarians to increase arterial oxygenation in anaesthetised animals, and in immobilized rhinoceros it has been shown to positively influence P_aO_2 (Bush et al. 2004; Heard et al. 1992). Paradoxically, perhaps owing to logistical difficulties, oxygen supplementation is not used commonly in current field immobilization protocols. Also, some veterinarians suggest that oxygen supplementation on its own appears to reduce respiratory rate (Buss, personal communication). It is therefore necessary to critically evaluate which methods, or combination of methods, currently available and used in the field, offer the best physiological support for the anaesthetized white rhinoceros. I used white rhinoceros in a controlled boma setting to critically compare and evaluate the administration of butorphanol, oxygen insufflation and the combination of these two interventions in the treatment of etorphine-induced respiratory depression. After determining the best available treatment in boma-immobilized rhinoceros, I evaluated that same treatment in field-immobilized white rhinoceros where increased exertion and psychological stress affect the physiology of the immobilized rhinoceros.

A caveat to the use of readily available mixed opioid agonist-antagonists is that they may reduce the sedative and immobilizing effect of the opioid agonist when combined in a dart mixture, thus prolonging induction times with a resultant increase in physiological stress.

It is therefore necessary to investigate ways of preventing opioid-induced respiratory depression without interfering with the opioid's ability to induce sedation and catatonic immobilization. Ideally, a drug found to attenuate opioid-induced respiratory depression should not prolong immobilization induction and it should be compatible with other agents so that it can be combined in a dart mixture to take effect in the period immediately after the animals become recumbent. Respiratory depression is most severe during this early period, which is generally before a veterinarian is able to intervene (Meyer et al. 2010).

Therefore, future pharmacological developments for the treatment and prevention of opioid-induced respiratory depression should focus on agents that reverse opioid-induced respiratory compromise through actions on non-opioid receptors. With an increased understanding of respiratory rhythm generation within the pre-Bötzinger complex, it is now possible to identify agents that may modulate respiratory rhythm without compromising opioid-induced sedation and immobilization. The network of neurons comprising the pre-Bötzinger complex depends on a number of neurotransmitters, including serotonin (5-HT) and glutamate, for the modulation of respiratory rhythm (Mironov & Richter 2000; Ramirez et al. 1997). Therefore, manipulating neuronal transmitter systems within the pre-Bötzinger complex may restore respiratory drive that has been dampened by opioid administration.

Indeed, serotonergic agonists already have shown promise in treating opioid-induced hypoxaemia in etorphine-immobilized ungulates (Meyer et al. 2006; Meyer et al. 2010). To improve the efficacy of serotonergic agents and to reduce potential side-effects, agents with greater specificity for the receptors involved in counteracting opioid-induced respiratory compromise need to be identified. The serotonergic drugs currently available are unfortunately not suitable for everyday wildlife work.

Glutamate, acting via AMPA receptors, is another critical component for the generation of respiratory rhythm within the pre-Bötzinger complex (Funk et al. 1993; Ge & Feldman 1998; Greer et al. 1991). Ampakines modulate AMPA receptors and have been shown to protect against opioid-induced respiratory depression in rodent models (Ren et al. 2006; Ren et al. 2009). Significantly, the ampakine, CX717, did not suppress opioid-induced analgesia in rats (Ren et al. 2009) or humans (Oertel et al. 2010). However these

molecules have never been tested in an ungulate model. I therefore evaluated a water-soluble ampakine, CX1942, in etorphine-immobilized goats to assess if these agents may provide an alternative solution to the treatment of opioid-induced respiratory depression in immobilized wildlife.

My interest in wildlife conservation together with applied physiology led me to investigate some of the pathophysiological events that occur during opioid-induced immobilization, particularly in one of our most vulnerable species, the white rhinoceros. Not only is the white rhinoceros particularly vulnerable to the uncontrollable rise in poaching, but for reasons not yet fully understood, this species is also particularly susceptible to opioid-induced respiratory depression. I have chosen to focus on opioid-induced respiratory depression because mitigating this severe consequence of chemical immobilization will decrease the risk of morbidity and mortality in many wildlife species, and in particular the white rhinoceros, in which more invasive and prolonged immobilization procedures are needed as the fight against poaching intensifies. While my thesis has focused on the chemical immobilization of wildlife, the findings also may be relevant to human medicine, where opioid-induced respiratory depression is a significant cause of mortality (Okie 2010). Below I outline how each chapter takes a step forward in the quest to treat etorphine-induced respiratory depression in immobilized herbivores.

Chapter 2: Nalbuphine and butorphanol reverse opioid-induced respiratory compromise but increase arousal in etorphine-immobilized goats (Capra hircus)

In human medicine, the mixed opioid agonist-antagonist nalorphine was one of the first agents used to treat morphine-induced respiratory depression. The finding that certain opioid ligands can elicit both agonistic and antagonistic effects at different opioid receptors led to the presumption that opioid-induced respiratory depression could be separated from opioid-induced analgesia and catatonia. Butorphanol, a readily available mixed opioid agonist-antagonist, is therefore currently used in white rhinoceros immobilization protocols in an attempt to improve respiration without affecting immobilization (Burroughs et al. 2012a). However, recent studies have shown that butorphanol may not be an effective mu-opioid receptor antagonist (Miller et al. 2013). I therefore set out to compare butorphanol to nalbuphine, another mixed opioid agonist-antagonist, to determine whether nalbuphine may be more efficacious in reversing etorphine-induced respiratory compromise, than is butorphanol. I evaluated these

compounds in etorphine-immobilized goats in a laboratory setting. Goats provide an ideal model for wild ungulates as they are easy to handle and habituate, and they have been used before as a successful model for the effects of opioid immobilization on cardiorespiratory function in wild ungulates (Buss & Meltzer 2001; Heard et al. 1990; Heard et al. 1996; Meyer et al. 2006). The physiological responses to administered drugs can therefore be evaluated without the background effect of a severe stress response that accompanies the capture of wild animals. I used an exercise physiology system to accurately measure respiratory rate, tidal volume, ventilation, oxygen consumption and carbon dioxide production continuously throughout the immobilization process. These data, together with regular arterial blood gas analyses allowed us to accurately assess how the drugs influenced the respiratory physiology of the goats.

Chapter 3: Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (Ceratotherium simum)

After establishing that nalbuphine was no better than butorphanol in its ability to reverse etorphine-induced respiratory depression in immobilized ungulates, I attempted to find the best currently available treatment of opioid-induced respiratory depression for white rhinoceros. The majority of protocols for white rhinoceros immobilization have been based on anecdotal evidence, or evidence from field trials, which are extremely difficult, if not impossible, to standardize. I therefore evaluated three treatments: butorphanol alone, oxygen supplementation alone, and butorphanol combined with oxygen, administered to the rhinoceros after they became recumbent. I tested these treatments in a cross-over trial in boma-habituated rhinoceros so that the treatments could be accurately compared with minimum confounding variability. I took arterial blood gas analyses for the accurate assessment of the rhinoceros' respiratory physiology throughout the immobilization period. I also continuously assessed the level of immobilization to determine whether the treatments had an effect on the sedative responses of etorphine.

Chapter 4: Improved cardiorespiratory function in immobilised wild white rhinoceros (Ceratotherium simum)

In southern Africa, the majority of wildlife, and in particular rhinoceros, immobilizations are carried out in free-ranging conditions with the aid of a helicopter. It was therefore a natural progression to test the best currently available protocol for the treatment of opioid-induced respiratory depression, as found in our boma study, in field-immobilized

rhinoceros where the increased exertion and sympathetic stress response would undoubtedly affect the physiology of captured rhinoceros. We standardized the field protocol as far as possible and followed the same procedure as that in the boma trial so that the results could be accurately evaluated and compared. Similar to the boma study, regular arterial blood gas analyses were conducted throughout the immobilization period and the level of immobilization was scored at regular intervals.

Chapter 5: Ampakine CX1942 attenuates opioid-induced respiratory depression and corrects hypoxaemia in etorphine-immobilized goats (Capra hircus)

In the previous chapters I have shown that administering butorphanol and supplying oxygen insufflation to the immobilized rhinoceros can significantly improve the safety of white rhinoceros immobilization. Although this protocol improves the oxygenation of the immobilized rhinoceros, it is not yet the ideal solution. We also found that rhinoceros were severely hypoxaemic as soon as they went down. Ideally, we need to find an agent that can be combined in the dart with the immobilizing cocktail to prevent, rather than treat opioid-induced respiratory depression. Partial-opioid agonists, such as butorphanol may delay the onset of immobilization and therefore are not ideal for the co-administration with etorphine. Promising advances in reversing opioid-induced respiratory depression without affecting the intended use of opioids have been made using ampakines (Lorier et al. 2010; Oertel et al. 2010; Ren et al. 2006; Ren et al. 2009; Ren et al. 2012). However, to date, nothing is known about the effects of ampakines in ungulates and whether they would be suitable for wildlife work. I therefore investigated the respiratory effects of a water-soluble ampakine, CX1942, and compared it to the known respiratory stimulant, doxapram, in etorphine-immobilized goats. I measured blood gases, respiratory rates, ventilation, tidal volumes, oxygen consumption and carbon dioxide production. I also assessed the quality of immobilization to determine if the ampakine affected the etorphine-induced immobilization.

References:

Alford BT, Burkhart RL & Johnson WP (1974) Etorphine and diprenorphine as immobilizing and reversing agents in captive and free-ranging mammals. *Journal of the American Veterinary Medical Association* 164:702-705.

Alibhai SK, Jewell ZC & Towindo SS (2001) Effects of immobilization on fertility in black rhino (*Diceros bicornis*). *Journal of the Zoological Society of London* 253:333-345.

Arai AC, Xia YF & Suzuki E (2004) Modulation of AMPA receptor kinetics differentially infuences synaptic plasticity in the hippocampus. *Neuroscience* 123:1011-1024.

Arnemo JM, Ahlqvist A, Andersen R, Berntsen F, Ericsson G, Odden J, Brunberg S, Segerström P & Swenson JE (2006) Risk of capture-related mortality in large free-ranging mammals: experiences from Scandinavia. *Wildlife Biology* 12:109-113.

Ballanyi K, Lalley PM, Hoch B & Richter DW (1997) cAMP-dependent reversal of opioid- and prostaglandin-mediated depression of the isolated respiratory network in newborn rats. *Journal of Physiology* 504:127-134.

Beaver WT & Feise GA (1978) A comparison of the analgesic effect of intramuscular nalbuphine and morphine in patients with postoperative pain. *The Journal of Pharmacology and Experimental Therapeutics* 204:487-496.

Benthuysen JL, Smith NT & Sanford TJ (1986) Physiology of alfentanil-induced rigidity. *Anesthesiology* 64:440-446.

Bianchi AL & Gestreau C (2009) The brainstem respiratory network: an overview of half a century of research. *Respiratory Physiology and Neurobiology* 168:4-12.

Bleul U, Bircher B, Jud RS & Kutter APN (2010) Respiratory and cardiovascular effects of doxapram and theophylline for the treatment of asphyxia in neonatal calves. *Theriogenology* 73:612-619.

Bonham AC (1995) Neurotransmitters in the CNS control of breathing. *Respiration Physiology* 101:219-230.

Boom M, Niesters M, Sarton E, Aarts L, Smith TW & Dahan A (2012) Non-analgesic effects of opioids: opioid-induced respiratory depression. *Current Pharmaceutical Design* 18:5994-6004.

Bowdle TA, Greichen SL, Bjurstrom RL & Schoene RB (1987) Butorphanol improves CO_2 response and ventilation after fentanyl anesthesia. *Anesthesia & Analgesia* 66:517-22.

Bowdle TA (1998) Adverse effects of opioid agonists and agonists-antagonists in anaesthesia. *Drug Safety* 19:173-189.

Burroughs R, Hofmeyr M, Morkel P, Kock MD, Kock R & Meltzer D (2012a) Chemical immobilization-individual species requirements. In: *Chemical and Physical Restraint of Wild Animals* (2nd edition). Kock MD & Burroughs R (eds.). IWVS (Africa), Grayton, South Africa, pp. 143-264.

Burroughs R, Meltzer D & Morkel P (2012b) Applied Pharmacology. In: *Chemical and Physical Restraint of Wild Animals* (2nd edition). Kock MD & Burroughs R (eds.). IWVS (Africa), Grayton, South Africa, pp. 53-80.

Burton MD & Kazemi H (2000) Neurotransmitters in central respiratory control. *Respiration Physiology* 122:111-121.

Bush M, Raath JP & Klein L (2004) Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen. *Journal of the South African Veterinary Association* 75:79-84.

Butelman ER, Winger G, Zernig G & Woods J (1995) Butorphanol: Characterization of agonist and antagonist effects in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics* 272:845-53.

Cattet M, Boulanger J, Stenhouse G, Powell RA & Reynolds-Hogland ML (2008) An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy* 89:973-990.

Cheney CS & Hattingh J (1987) Effects of chemical immobilization on the blood composition of impala. *Journal of the South African Veterinary Association* 59:13-18.

Commiskey S, Fan L-W, Ho IK & Rockhold RW (2005) Butorphanol: effects of a prototypical agonist-antagonist analgesic on κ-opioid receptors. *Journal of Pharmacological Sciences* 98:109-116.

Dahan A, Sarton E, Teppema L, Olievier C, Nieuwenhuijs D, Matthes HWD & Kieffer BL (2001) Anesthetic potency and influence of morphine and sevoflurane on respiration in μ-opioid receptor knockout mice. *Anesthesiology* 94:824-32.

Dahan A & Teppema LJ (2003) Influence of anaesthesia and analgesia on the control of breathing. *British Journal of Anaesthesia* 91:40-49.

Dahan A (2007) Novel data on opioid effect on breathing and analgesia. *Seminars in Anesthesia, Perioperative Medicine and Pain* 26:58-64.

Dahan A, Aarts L & Smith TW (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 112:226-38.

Dobrenis K, Makman MH & Stefano GB (1995) Occurrence of the opiate alkaloid-selective μ_3 receptor in mammalian microglia, astrocytes and Kupffer cells. *Brain Research* 686:239-248.

Dutschmann M, Waki H, Manzke T, Simms AE, Pickering AE, Richter DW & Paton JFR (2009) The potency of different serotonergic agonists in counteracting opioid evoked cardiorespiratory disturbances. *Philosophical Transactions of The Royal Society B* 364:2611-2623.

Dyck MG, Soon W, Baydack RK, Legates DR, Baliunas S, Ball TF & Hancock LO (2007) Polar bears of western Hudson Bay and climate change: Are warming spring air temperatures the "ultimate" survival control factor? *Ecological Complexity* 4:73-84.

Erlichman JS & Leiter JC (2010) Glia modulation of the extracellular milieu as a factor in central CO_2 chemosensitivity and respiratory control. *Journal of Applied Physiology* 108:1803-1811.

Fahlman Å (2008). Advances in wildlife immobilization and anaesthesia: clinical and physiological evaluation in selected species. Ph.D. thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Fahlman Å, Caulkett N, Arnemo JM, Neuhaus P & Ruckstuhl KE (2012) Efficacy of a portable oxygen concentrator with pulsed delivery for treatment of hypoxemia during anesthesia of wildlife. *Journal of Zoo and Wildlife Medicine* 43:67-76.

Fahlman Å (2014) Oxygen Therapy In: *Zoo Animal and Wildlife Immobilization and Anesthesia* (2nd edition). West G, Heard D & Caulkett N (eds.). John Wiley & Sons, Inc., Iowa, USA. pp. 69-81.

Fahlman Å, Arnemo JM, Pringle J & Nyman G (2014) Oxygen supplementation in anesthetized brown bears (*Ursus arctos*)-How low can you go? *Journal of Wildlife Diseases* 50:574-581.

Feldman JL, Mitchell GS & Nattie EE (2003) Breathing: rhythmicity, plasticity, chemosensitivity. *Annual Review of Neuroscience* 26:239-266.

Feldman JL & Del Negro CA (2006). Looking for inspiration: new perspectives on respiratory rhythm. *Nature Reviews Neuroscience* 7:232.doi:10.1038/nrn1871

Ferguson LM & Drummond GB (2006) Acute effects of fentanyl on breathing pattern in anaesthetized subjects. *British Journal of Anaesthesia* 96:384-390.

Forster HV, Martino P, Hodges M, Krause K, Bonis J, Davis S & Pan L (2008) The carotid chemoreceptors are a major determinant of ventilator CO_2 sensitivity and of $PaCO_2$ during eupneic breathing. *Advances in Experimental Medicine and Biology* 605:322-326.

Fredholm BB, Battig K, Holmen J, Nehlig A & Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews* 51:83-133.

Funk G, Smith J & Feldman J (1993) Generation and transmission of respiratory oscillations in medullary slices: role of excitatory amino acids. *Journal of Neurophysiology* 70:1497-1515.

Ganong WF (ed.) (2005) *Review of Medical Physiology* (22nd edition). McGraw-Hill Companies, Inc., USA, pp. 671-685.

Ge Q & Feldman JL (1998) AMPA receptor activation and phosphatase inhibition affect neonatal rat respiratory rhythm generation. *Journal of Physiology* 509:255-266.

Gold SJ, Ambros-Ingerson J, Horowitz JR, Lynch G & Gall CM (1997) Stoichiometries of AMPA receptor subunit mRNAs in rat brain fall into discrete categories. *Journal of Comparative Neurology* 385:491-502.

Golder FJ, Hewitt MM & McLeod JF (2013) Respiratory stimulant drugs in the post-operative setting. *Respiratory Physiology and Neurobiology* 189:395-402.

Greer JJ, Smith JC & Feldman JL (1991) Role of excitatory amino acids in the generation and transmission of respiratory drive in neonatal rat. *Journal of Physiology* 437:727-749.

Grimm KA & Lamont LA (2007) Clinical pharmacology, In: *Zoo Animal and Wildlife Immobilization and Anesthesia*, West G, Heard D & Caulkett N (eds.). Blackwell Publishing Inc. Ames, Iowa, USA. pp. 3-36.

Guenther U, Manzke T, Wrigge H, Dutschmann M, Zinserling J, Putensen C & Hoeft A (2009) The counteraction of opioid-induced ventilatory depression by the serotonin 1_A-agonist 8-OH-DPAT does not antagonize antinociception in rats *in situ* and *in vivo*. *Anesthesia & Analgesia* 108:1169-1176.

Guenther U, Theuerkauf NU, Huse D, Boettcher MF, Wensing G, Putensen C & Hoeft A (2012) Selective 5-HT_{1A}-R-agonist repinotan prevents remifentanil-induced ventilatory depression and prolongs antinociception. *Anesthesiology* 116:56-64.

Gutstein HB & Akil H (2001) Opioid Analgesics. In: *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (10th edition). Hardman JG & Limbirg LE (eds.). McGraw-Hill Medical Publishing Division, USA, pp 569-619.

Haigh JC (1990) Opioids in zoological medicine. *Journal of Zoo and Wildlife Medicine* 21:391-413.

Haji A, Takeda R & Okazaki M (2000) Neuropharmacology of control of respiratory rhythm and pattern in mature mammals. *Pharmacology & Therapeutics* 86:277-304.

Hajiha M, DuBord MA, Liu H, Horner RL (2009) Opioid receptor mechanisms at the hypoglossal motor pool and effects on tongue muscle activity *in vivo*. *Journal of Physiology* 587:2677-2692.

Harthoorn AM & Bligh J (1965) A new oripavine derivative with potent morphine-like properties for the restraint of the large wild African mammals. *Research in Veterinary Science* 6:290-299.

Harthoorn AM (1966) Restraint of undomesticated animals. *Journal of the American Veterinary Medical Association* 149:875-80.

Heard DJ, Kollias GV, Buss D, Caligiuri R & Coniglario J (1990) Comparative cardiovascular effects of intravenous etorphine and carfentanil in domestic goats. *Journal of Zoo and Wildlife Medicine* 21:166-170.

Heard DJ, Olsen H & Stover J (1992) Cardiopulmonary changes associated with chemical immobilization and recumbency in white rhinoceros (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 23:197-200.

Heard DJ, Nichols WW, Buss D & Kollias GV (1996) Comparative cardiopulmonary effects of intramuscularly administered etorphine and carfentanil in goats. *American Journal of Veterinary Research* 57:87-96.

Holden JE, Jeong Y & Forrest JM (2005) The endogenous opioid system and clinical pain management. *AACN Clinical Issues* 16:291-301.

Hoskins PJ & Hanks GW (1991) Opioid agonist-antagonist drugs in acute and chronic pain states. *Drugs* 41:326-344.

Howard P (1989) Hypoxia, almitrine, and peripheral neuropathy. *Thorax* 44:247-50.

Hülsmann S, Oku Y, Zhang W & Richter DW (2000) Metabolic coupling between glia and neurons is necessary for maintaining respiratory activity in transverse medullary slices of neonatal mouse. *European Journal of Neuroscience* 12:856-862.

Hutchinson MR, Northcutt AL, Chao LW, Kearney JJ, Zhang Y, Berkelhammer DL, Loram LC, Rozeske RR, Bland ST, Maier SF, Gleeson TT & Watkins LR (2008) Minocycline suppresses morphine-induced respiratory depression, suppresses morphine-induced reward, and enhances systemic morphine-induced analgesia. *Brain, Behavior, and Immunity* 22:1248-1256.

Huxtable RJ & Schwarz SKW (2001) The isolation of morphine-first principles in science and ethics. *Molecular interventions* 1:189-191.

Inoue M, Kobayashi M, Kozaki S, Zimmer A & Ueda H (1998) Nociceptin/orphanin FQ-induced nociceptive responses through substance P release from peripheral nerve endings in mice. *Proceedings of the National Academy of Sciences of the United States of America* 95:10949-10953.

Inturrisi CE (2002) Clinical pharmacology of opioids for pain. *Clinical Journal of Pain* 13:S1-S11.

Janczewski WA & Feldman JL (2006) Distinct rhythm generators for inspiration and expiration in the juvenile rat. *Journal of Physiology* 570:407-420.

Johnson RA & De Morias HA (2012) Respiratory acid-base disorders. In: *Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice* (4th edition). DiBartola SP (ed.). St Louis, Elsevier Saunders, pp. 287-301.

Kieffer BL (1999) Opioids: first lessons from knock-out mice. *Trends in Pharmacological Sciences* 20:19-26.

Kieffer BL & Gavériaux-Ruff C (2002) Exploring the opioid system by gene knock-out. *Progress in Neurobiology* 66:285-306.

Kim H & Suh Y (2009) Minocycline and neurodegenerative diseases. *Behavioural Brain Research* 196:168-179.

Kisling VN (2001) *Zoo and Aquarium History: Ancient Animal Collections to Zoological Gardens*. CRC Press, Taylor & Francis Group, LLC, USA, pp.1-42.

Knottenbelt MK (1990) Causes of mortality in impala (*Aepyceros melampus*) on 20 game farms in Zimbabwe. *Veterinary Record* 127:47-50.

Kock MD, Clark RK, Franti CE, Jessup DA & Wehausen JD (1987) Effects of capture on biological parameters in free-ranging bighorn (*Ovis Canadensis*): evaluation of normal, stressed and mortality outcomes and documentation of postcapture survival. *Journal of Wildlife Diseases* 23:652-662.

Kock MD, La Grange M & Du Toit R (1990) Chemical immobilization of free-ranging black rhinoceros (*Diceros bicornis*) using combinations of etorphine (M99), fentanyl, and xylazine. *Journal of Zoo and Wildlife Medicine* 21:155-165.

Kock MD, Morkel P, Atkinson M & Foggin C (1995) Chemical immobilization of free-ranging white rhinoceros (*Ceratotherium simum simum*) in Hwange and Matobo National Parks, Zimbabwe, using combinations of etorphine (M99), fentanyl, xylazine and detomidine. *Journal of Zoo and Wildlife Medicine* 26:207-219.

Laffey JG & Kavanagh BP (2002) Hypocapnia. *New England Journal of Medicine* 347:43-53.

Lalley PM (2005) D1-dopamine receptor agonists prevent and reverse opiate depression of breathing but not antinociception in the cat. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 289:R45-R51.

Lalley PM (2008) Opioidergic and dopaminergic modulation of respiration. *Respiratory Physiology and Neurobiology* 164:160-167.

Lam A & Newhouse MT (1990) Management of asthma and chronic airflow limitation. Are methylxanthines obsolete? *Chest* 98:44-52.

Larsen DG & Gauthier DA (1989) Effects of capturing pregnant moose and calves on calf survivorship. *The Journal of Wildlife Management* 53:564-567.

Laubie M & Schmitt H (1980) Long-lasting hyperventilation induced by almitrine: evidence for a specific effect on carotid and thoracic chemoreceptors. *European Journal of Pharmacology* 61:125-136.

Lian M, Evans AL, Bertelsen MF, Fahlman A, Haga HA, Ericcson G & Arnemo JM (2014) Improvement of arterial oxygenation in free-ranging moose (*Alces alces*) immobilised with etorphine-acepromazine-xylazine. *Acta Veterinaria Scandinavia* 56:51-66.

Ling GSF, Spiegel K, Lockhart SH & Pasternak GW (1985) Separation of opioid analgesia from respiratory depression: evidence for different receptor mechanisms. *The Journal of Pharmacology and Experimental Therapeutics* 232:149-155.

Lonergan T, Goodchild AK, Christie MJ & Pilowsky PM (2003) Mu opioid receptors in rat ventral medulla: effects of endomorphine-1 on phrenic nerve activity. *Respiratory Physiology & Neurobiology* 138:165-178.

Lorier AR, Funk GD & Greer JJ (2010) Opiate-induced suppression of rat hypoglossal motoneuron activity and its reversal by ampakine therapy. *PLoS ONE* 5:e8766.

Lötsch J, Skarke C, Schneider A, Hummel T, Geisslinger G (2005) The 5-hydroxy-tryptamine 4 receptor agonist mosapride does not antagonize morphine-induced respiratory depression. *Clinical Pharmacology & Therapeutics* 78:278-287.

Lumb AB (ed.) (2005a) Control of breathing. In: *Nunn's Applied Respiratory Physiology* (6th edition). Elsevier Butterworth Heinemann, Philadelphia, USA, pp. 55-75.

Lumb AB (ed.) (2005b) The pulmonary circulation. In: *Nunn's Applied Respiratory Physiology* (6th edition). Elsevier Butterworth Heinemann, Philadelphia, USA, pp. 92-109.

Lynch G (2004) AMPA receptor modulators as cognitive enhancers. *Current Opinion in Pharmacology* 4:4-11.

Lynch G (2006) Glutamate-based therapeutic approaches: ampakines. *Current Opinion in Pharmacology* 6:82-88.

Magosso E, Ursino M & van Oostrom JH (2004) Opioid-induced respiratory depression: A mathematical model for fentanyl. *IEEE Transactions on Biochemical Engineering* 51:1115-1128.

Manzke T, Ponimaskin EG, Haller M, Dutschmann M, Richter DW, Guenther U & Schwarzacher S (2003) 5-$HT_{4(a)}$ receptors avert opioid-induced breathing depression without loss of analgesia. *Science* 301:226-229.

Martin LJ, Blackstone CD, Levey AI, Huganir RL & Price DL (1993) AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience* 53:327-358.

May CN, Dashwood MR, Whitehead CJ & Mathias CJ (1989) Differential cardiovascular and respiratory responses to central administration of selective opioid agonists in conscious rabbits: correlation with receptor distribution. *British Journal of Pharmacology* 98:903-913.

McDonald J & Lambert DG (2005) Opioid receptors. *Continuing Education in Anaesthesia, Critical Care & Pain* 5:22-25.

Mellen N, Janczewski WA, Bocchiaro CM & Feldman JL (2003) opioid-induced quantal slowing reveals dual networks for respiratory rhythm generation. *Neuron* 37:821-826.

Meltzer D & Kock N (2012) Stress and capture-related death. In: *Chemical and Physical restrain of wild animals* (2nd edition). Kock MD & Burroughs R (eds.). IWVS (Africa), Greyton, South Africa pp 81-88.

Meyer LCR, Fuller A & Mitchell D (2006) Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 290:R405-R413.

Meyer LCR, Hetem RS, Fick LG, Mitchell D & Fuller A (2010) Effects of serotonin agonists and doxapram on respiratory depression and hypoxemia in etorphine-immobilized impala (*Aepyceros melampus*). *Journal of Wildlife Diseases*, 46:514-524.

Meyer LCR, Hetem R, Mitchell D & Fuller A (2015) Hypoxia following etorphine administration in goats (*Capra hircus*) results more from pulmonary hypertension than from hypoventilation. *BMC Veterinary Research* 11:18.

Mich PM, Wolfe LL, Sirochman TM, Sirochman MA, Davis TR, Lance WR & Miller MW (2008) Evaluation of intramuscular butorphanol, azaperone, and medetomidine and nasal oxygen insufflation for the chemical immobilization of white-tailed deer, *Odocoileus virginianus. Journal of Zoo and Wildlife Medicine* 39:480-487.

Miller M, Buss P, Joubert J, Mathebula M, Kruger M, Martin L, Hofmeyr M & Olea-Popelka F (2013) Use of butorphanol during immobilization of free-ranging white rhinoceros (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 44:55-61.

Mironov S & Richter D (2000) Intracellular signalling pathways modulate K_{ATP} channels in inspiratory brainstem neurons and their hypoxic activation: involvement of metabotropic receptors, G-proteins and cytoskeleton. *Brain Research* 853:60-67.

Montandon G, Qin W, Liu H, Ren J, Greer JJ & Horner RL (2011) PreBötzinger complex neurokinin-1 receptor-expressing neurons mediate opioid-induced respiratory depression. *The Journal of Neuroscience* 31:1292-1301.

Neumann W, Ericsson G, Dettki H & Arnemo JM (2011) Effect of immobilizations on the activity and space use of female moose (*Alces alces*) *Canadian Journal of Zoology* 89:1013-1018.

Nielsen L (1999) *Chemical Immobilization of Wild and Exotic animals*. Iowa State University Press, Ames, Iowa, USA. pp. 3-12.

Nishii Y, Okada Y, Yokoba M, Katagiri M, Yanaihara T, Masuda N, Easton PA & Abe T (2008) Aminophylline increases parasternal intercostal muscle activity during hypoxia in humans. *Respiratory Physiology and Neurobiology* 161:69-75.

Oertel BG, Schneider A, Rohrbacher M, Schmidt H, Tegeder I, Geisslinger G & Lötsch J (2007) The partial 5-hydroxytryptamine 1A receptor agonist buspirone does not antagonize morphine-induced respiratory depression in humans. *Clinical Pharmacology & Therapeutics* 81:59-68.

Okie S (2010) A flood of opioids, a rising tide of deaths. *New England Journal of Medicine* 363:1981-1985.

Ostadal B, Ostadalova I & Dhalla NS (1999) Development of cardiac sensitivity to oxygen deficiency: comparative and ontogenetic aspects. *Pharmacological Reviews* 79:635-659.

Pace RW, Mackay DD, Feldman JL & Del Negro CA (2007) Inspiratory bursts in the preBötzinger complex depend on a calcium-activated non-specific cation current linked to glutamate receptors in neonatal mice. *The Journal of Physiology* 582:113-125.

Pallasch TJ & Gill CJ (1985) Butorphanol and nalbuphine: a pharmacologic comparison. *Oral Surgery, Oral Medicine, and Oral Pathology* 59:15-20.

Pasternak G & Pan Y (2011) Mu opioid receptors in pain management. *Acta Anaesthesiologica Taiwanica* 49:21-25.

Pasternak G (2014) Opioids and their receptors: are we there yet? *Neuropharmacology* 76:198-203.

Pattinson KTS (2008) Opioids and the control of respiration. *British Journal of Anaesthesia* 100:747-758.

Picone S, Aufieri R & Paolillo P (2014) Apnea of prematurity: challenges and solutions. *Research and Reports in Neonatology* 4:101-109.

Player I (1967) Translocation of white rhinoceros in South Africa. *Oryx – The International Journal of Conservation* 9:137-150.

Player I (1993) Foreword. In: *The Capture and Care Manual*. McKenzie AA (ed.). Wildlife Decision Support Services cc and The South African Veterinary Foundation Pretoria, South Africa. pp. vii-xiii.

Plumb DC (ed.) (2002) *Veterinary Drug Handbook* (4th edition), Iowa State Press, Iowa, USA. pp. 294-295.

Portas TJ (2004) A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species. *Australian Veterinary Journal* 82:542-549.

Ramirez J & Richter DW (1996) The neuronal mechanisms of respiratory generation. *Current Opinion in Neurobiology* 6:817-825.

Ramirez JM, Telgkamp P, Elsen FP, Quellmalz UJA & Richter DW (1997) Respiratory rhythm generation in mammals: synaptic and membrane properties. *Respiratory Physiology* 110:71-85.

Ren J, Poon BY, Tang Y, Funk GD & Greer JJ (2006) Ampakines alleviate respiratory depression in rats. *American Journal of Respiratory and Critical Care Medicine* 174:1384-1391.

Ren J, Ding X, Funk GD & Greer JJ (2009) Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. *Anesthesiology* 110:1364-1370.

Richter DW, Mironov SL, Büsselberg D, Lalley PM, Bischoff AM & Wilken B (2000) Respiratory rhythm generation: plasticity of a neuronal network. *The Neuroscientist* 6:181-198.

Richter DW & Spyer KM (2001) Studying rhythmogenesis of breathing: comparison of *in vivo* and *in vitro* models. *TRENDS in Neuroscience* 24:464-472.

Richter DW, Manzke T, Wilken B & Ponimaskin E (2003) Serotonin receptors: guardians of stable breathing. *Trends in Molecular Medicine* 9:542-548.

Rogers PS (1993) Chemical capture of the white rhinoceros *Ceratotherium simum.* In: *The Capture and Care Manual*. McKenzie AA (ed.). Wildlife Decision Support Services and The South African Veterinary Foundation, South Africa pp. 512-528.

Romagnoli A & Keats AS (1980) Ceiling effect for respiratory depression by nalbuphine. *Clinical Pharmacology & Therapeutics* 27:478-485.

Roozekrans M, Van der Schrier R, Okkerse P, Hay J, McLeod J & Dahan A (2014) Two studies on reversal of opioid-induced respiratory depression by BK-channel blocker GAL021 in human volunteers. *Anesthesiology* 121:459-68.

Roozekrans M, Olofsen E, Van der Schrier R, van Gerven J, Peng S, McLeod J & Dahan A (2015) Reversal of opioid-induced respiratory depression by BK-channel blocker GAL021: A pharmacokinetic-pharmacodynamic modeling study in healthy volunteers. *Clinical Pharmacology & Therapeutics* 97:641-9.

Roquebert J & Delgoulet C (1988) Cardiovascular effects of etorphine in rats. *Journal of Autonomic Pharmacology* 8:39-43.

Rosenblum A, Marsch LA, Joseph H & Portenoy RK (2008) Opioids and the treatment of chronic pain: controversies, current status, and future directions. *Experimental and Clinical Psychopharmacology* 16:405-416.

Sahibzada N, Ferreira M, Wasserman AM, Taveira-Dasilva AM & Gillis RA (2000) Reversal of morphine-induced apnea in the anesthetized rat by drugs that activate 5-hydroxytryptamine(1A) receptors. *Journal of Pharmacology and Experimental Therapeutics* 292:704-713.

Santiago TV & Edelman EH (1985) Opioids and breathing. *Journal of Applied Physiology* 59:1675-1685.

Savarie PJ (1976) Pharmacological review of chemicals used for the capture of animals. *Proceedings of the 7th Vertebrate Pest Conference*, 3 January 1976. Paper 41. http://digitalcommons.unl.edu/vpc7/41

Schmidt C, Bellingham MC & Richter DW (1995) Adenosinergic modulation of respiratory neurones and hypoxic responses in the anaesthetized cat. *Journal of Physiology* 483:769-781.

Schmidt WK, Tam SW, Schotzberger GS, Smith DH, Clark R & Vernier VG (1985) Nalbuphine. *Drug and Alcohol Dependence* 14:339-362.

Schumacher J (2008) Side effects of etorphine and carfentanil in nondomestic hoofstock. In: *Zoo and Wild Animal Medicine* (volume 6). Fowler ME & Miller RE (eds.). Saunders Elsevier, St. Louis, Missouri. pp. 455-461.

Schwarzacker SW, Smith JC & Richter DW (1995) Pre-Bötzinger complex in the cat. *Journal of Neurophysiology* 73:1452-61.

Shang Y & Filizola M (2015) Opioid receptors: structural and mechanistic insights into pharmacology and signaling. *European Journal of Pharmacology* 763:206-213.

Sharma V, Dutt S, Kumar R, Sharma P, Javed A & Guleria R (2015) OPIOID pharmacology: a review. *International Journal of Scientific Research in Science & Technology* 1:1-11.

Shook JE, Watkins WD & Camporesi EM (1990) Differential roles of opioid receptors in respiration, respiratory disease, and opiate-induced respiratory depression. *American Review of Respiratory Disease* 142:895-909.

Smith JC, Ellenberger HH, Ballanyi K, Richter DW & Feldman JL (1991) Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* 254:726-729.

Smith JC, Abdala APL, Koizumi H, Rybak IA & Paton JFR (2007) Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms. *Journal of Neurophysiology* 98:3370-3387.

Smith JC, Abdala APL, Borgmann A, Rybak IA & Paton JFR (2013) Brainstem respiratory networks: building blocks and microcircuits. *Trends in Neuroscience* 36:152-162.

Spraker TR (1993) Stress and capture myopathy in artiodactylids. In: *Zoo & Wild Animal Medicine. Current Therapy 3*. Fowler ME (ed.). W.B. Saunders Company, London. pp. 481-488.

Spyer KM & Gourine AV (2009) Chemosensory pathways in the brainstem controlling cardiorespiratory activity. *Philosophical Transactions of the Royal Society B* 364:2603-2610.

Stefano GB, Digenis A, Spector S, Leung MK, Bilfinger TV, Makman MH, Scharrer B & Abumrad NN (1993) Opiate-like substances in an invertebrate, an opiate receptor on invertebrate and human immunocytes, and a role in immunosuppression. *Proceedings of the National Academy of Sciences of the United States of America* 90:11099-11103.

Stein C, Machelska H, Binder W & Schäfer M (2001) Peripheral opioid analgesia. *Current Opinion in Pharmacology* 1:62-65.

Stephen CR & Talton I (1964) Investigation of doxapram as a postanesthetic respiratory stimulant. *Anesthesia & Analgesia* 43:628-640.

Swan GE (1993) Drugs used for the immobilization, capture and translocation of wild animals. In: *The Capture and Care Manual.* McKenzie AA (ed.). Wildlife Decision Support Services and The South African Veterinary Foundation, South Africa pp. 3-98.

Takeda S, Eriksson LI, Yamamoto Y, Joensen H, Onimaru H & Lindahl SGE (2001) Opioid action on respiratory neuron activity of the isolated respiratory network in newborn rats. *Anesthesiology* 95:740-749.

Van der Schrier R, Roozekrans M, Van Velzen M, Dahan A & Niesters M (2014) Opioid-induced respiratory depression: reversal by non-opioid drugs. *F1000 Prime Reports* 6:79.

Van der Schrier R & Roozekrans M (2015) Historical overview of (non-opioid) reversal agends of opioid-induced respiratory depression (OIRD). *Nederlands Tijdschrift Voor Anesthesiology* 28:25-30.

Vankova ME, Weinger MB, Chen D-Y, Bronson B, Motis V & Koob GF (1996) Role of central mu, delta-1, and kappa-1 opioid receptors in opioid-induced muscle rigidity in the rat. *Anesthesiology* 85:574-583.

Vivian JA, DeYoung MB, Sumpter TL, Traynor JR, Lewis JW & Woods JH (1999) Kappa-opioid receptor effects of butorphanol in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics* 290:259-265.

Volkow ND, Frieden TR, Hyde PS & Cha SS (2014) Medication-assisted therapies – tackling the opioid overdose epidemic. *New England Journal of Medicine* 370:2063-2066.

Wenger S, Boardman W, Buss P, Govender D & Foggin C (2007) The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field-anaesthetized white rhinoceroses (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 38:380-387.

Wesensten NJ, Reichardt RM & Balkin TJ (2007) Ampakine (CX717) effects on performance and alertness during simulated night shift work. *Aviation, Space, and Environmental Medicine* 78:937-943.

West JB (2012) *Respiratory Physiology – the essentials* (9th edition). Lippincott Williams & Wilkins, Baltimore, USA.

Whiteside GT, Hummel M, Boulet J, Beyenhof JD, Strenkowski B, Dell John J, Knappenberger T, Maselli H & Koetzner L (2015) Robustness of arterial blood gas analysis for assessment of respiratory safety pharmacology in rats. *Journal of Pharmacological and Toxicological Methods* 78:32-41.

Wilken B, Ramirez JM, Hanefeld F & Richter DW (2000) Aminophylline modulation of the mouse respiratory network changes during postnatal maturation. *Journal of Applied Physiology* 89:2015-2022.

Williams ES & Thorne ET (1996) Exertional myopathy. In: *Noninfectious diseases of wildlife* (2nd edition). Fairbrother A, Locke LL & Hoff GL (eds.). Manson Publishing, London, UK, pp. 181-193.

Wilson WC & Benumof JL (2005) Respiratory physiology and respiratory function during anesthesia. In: *Miller's Anesthesia* (6th edition). Miller RD (ed.). Elsevier Churchill Livingstone, Philadelphia, pp. 679-718.

Yokoba M, Ichikawa T, Takakura A, Ishii N, Kurosaki Y, Yamada Y, Tsukushi T, Masuda N, Easton P & Katagiri M (2015) Aminophylline increases respiratory muscle activity during hypercapnia in humans. *Pulmonary Pharmacology and Therapeutics* 30:96-101.

Yost CS (2006) A new look at the respiratory stimulant doxapram. *CNS Drug Reviews* 12:236-249.

Zöllner C & Stein C (2007) Opioids. *Handbook of Experimental Pharmacology* 177:31-63.

Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA & Stohler CS (2001) Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science* 293:311-315.

Zucker JR, Neuenfeldt T & Freund PR (1987) Respiratory effects of nalbuphine and butorphanol in anesthetized patients. *Anesthesia & Analgesia* 66:879-81.

CHAPTER 2

Nalbuphine and butorphanol reverse opioid-induced respiratory compromise but increase arousal in etorphine-immobilized goats (*Capra hircus*).

Haw AJ, Meyer LCR & Fuller A. (2016) *Veterinary Anaesthesia and Analgesia.* In press doi:10.1111/vaa.12343

Veterinary Anaesthesia and Analgesia, 2016 doi:10.1111/vaa.12343

RESEARCH PAPER

Nalbuphine and butorphanol reverse opioid-induced respiratory depression but increase arousal in etorphine-immobilized goats (*Capra hircus*)

Anna J Haw*, Leith CR Meyer*,† & Andrea Fuller*,†

*Brain Function Research Group, Faculty of Health Sciences, School of Physiology, University of the Witwatersrand, Joahnnesburg, South Africa

†Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

Correspondence: Anna J Haw, Brain Function Research Group, Faculty of Health Sciences, School of Physiology, University of the Witwatersrand, 7 York Road, Parktown, Joahnnesburg 2193, South Africa. E-mail: anna.haw@wits.ac.za

Abstract

Objectives To evaluate and compare the efficacy of two opioid agonist-antagonists, nalbuphine and butorphanol, in reversing etorphine-induced respiratory depression in immobilized goats.

Study design Prospective, crossover, experimental trial conducted at 1753 m.a.s.l.

Animals Eight adult female Boer goats (*Capra hircus*).

Methods Eight minutes following immobilization with an intramuscular injection of 0.1 mg kg^{-1} etorphine, goats were given one of nalbuphine (0.8 mg kg^{-1}), butorphanol (0.1 mg kg^{-1}) or sterile water intravenously, in random order in three trials. Respiratory rate (f_R), ventilation, tidal volume, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were measured continuously. Arterial blood samples to determine PaO_2 and $PaCO_2$ were taken 2 minutes before and at 5 minute intervals after etorphine administration for 25 minutes.

Results Both nalbuphine and butorphanol increased mean PaO_2 from 44 mmHg (5.9 kPa) to 63 mmHg (8.4 kPa) after etorphine administration. Butorphanol, but not nalbuphine, also corrected hypopnea and hypoventilation such that f_R increased from 13 ± 4 to 21 ± 7 breaths $minute^{-1}$ (compared with 16 ± 6 breaths $minute^{-1}$ following nalbuphine) and ventilation increased from 4.69 ± 3.04 to 6.91 ± 4.42 L $minute^{-1}$ following butorphanol administration. Despite decreases in $PaCO_2$ following nalbuphine and butorphanol, $PaCO_2$ remained elevated compared with pre-immobilization values [nalbuphine: 34 ± 3 mmHg (4.5 ± 0.3 kPa); butorphanol: 34 ± 2 mmHg (4.5 ± 0.3 kPa)] throughout the immobilization. Both agents also decreased the level of immobilization, and increased $\dot{V}O_2$ and $\dot{V}CO_2$.

Conclusions Nalbuphine and butorphanol significantly improved respiratory function in immobilized goats, with butorphanol eliciting a greater positive response than nalbuphine. However, both opioid agonist-antagonists partly reversed etorphine-induced immobilization.

Clinical relevance Butorphanol and nalbuphine can be used to improve respiratory parameters in etorphine-immobilized wildlife, with butorphanol being more effective, but unwanted arousal can occur.

Keywords immobilization, mixed opioid agonist-antagonists, opioids, wildlife.

Introduction

Etorphine, a highly potent opioid agonist, is used extensively by wildlife veterinarians to bring about rapid and reliable immobilization, especially in large herbivores. Opioids have obvious advantages over any other class of drug in their ability to reduce pain and induce anaesthesia or immobilization in wildlife (Dahan et al. 2010; Burroughs et al. 2012). However, in both human anaesthesia and wildlife immobilization, opioids cause respiratory depression, which can be life-threatening (Bowdle 1998; Dahan et al. 2010; Burroughs et al. 2012).

Opioid ligands interact with three major opioid receptor subtypes: mu (μ), delta (δ) and kappa (κ) receptors. Interactions with μ-receptors are primarily responsible for respiratory depression, analgesia, catatonia and euphoria, whereas κ-receptor activation facilitates analgesia and sedation (Bowdle 1998; Kieffer 1999). Therefore, synthetic opioid agonist-antagonists with predominantly μ-antagonistic and κ-agonistic activities should produce analgesia and sedation with little or no respiratory depression (Bowdle 1998). Butorphanol and nalbuphine are two such opioid agonist-antagonists (Hoskin & Hanks 1991). In wildlife immobilization, butorphanol is used to reverse etorphine-induced respiratory depression, especially in opioid-sensitive species, such as the white rhinoceros (Burroughs et al. 2012). However, recent studies indicate that butorphanol is not an effective μ-antagonist in rhinoceros as was previously thought (Miller et al. 2013; Haw et al. 2014). Evidence from human and rodent studies indicates that nalbuphine, which is not currently used in veterinary medicine, may be a better μ-opioid antagonist than butorphanol (Pallasch & Gill 1985; Schmidt et al. 1985; Zucker et al. 1987).

In this study, we used the domestic goat as a model in which to compare and evaluate the effectiveness of butorphanol and nalbuphine in reversing opioid-induced respiratory depression in immobilized ungulates. The primary objective of the study was to determine whether nalbuphine differs from butorphanol in attenuating etorphine-induced respiratory depression without causing unwanted arousal. We achieved this objective by measuring physiological parameters including the partial pressures of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$), respiratory rate (f_R), tidal volume, ventilation, oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). We used habituated goats in order to reduce the confounding effects of a sympathetic stress response, which normally accompanies etorphine-induced immobilization. We used a scoring system based on movement to assess the level of immobilization.

Materials and methods

This project was approved by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance 2013/26/04) and conducted in adherence to the ARRIVE guidelines for reporting *in vivo* experiments (Kilkenny et al. 2010). We used eight healthy adult female goats (*Capra hircus*) with a mean ± standard deviation (SD) body mass of 27.1 ± 1.6 kg. Juvenile and pregnant animals were excluded and females were used for ease of handling. A sample size of eight was chosen based on the statistically significant and clinically relevant results from a previous controlled repeated-measures study in which eight animals were used in a similar protocol to measure cardiopulmonary parameters in etorphine-immobilized goats (Meyer et al. 2006). The goats were housed in temperature-controlled indoor pens in Johannesburg, South Africa, at an altitude of 1753 m, on a 12 hour light/dark cycle. Barometric pressure, measured by the onboard barometer of the blood gas analyser, lay in the range of 622.4–626.2 mmHg (83.0–83.5 kPa); the partial pressure of inspired oxygen therefore was about 131 mmHg (17.5 kPa). Over a period of 6 weeks, before the start of data collection, the goats were habituated to being manually restrained in sternal recumbency on a work table with a face mask over the muzzle.

Drugs

Etorphine hydrochloride (9.8 mg mL^{-1}; Captivon; Wildlife Pharmaceuticals Pty Ltd, South Africa) was injected intramuscularly (IM) into the right gluteal muscle (time 0) at a dose of 0.1 mg kg^{-1}. Eight minutes later, 0.1 mg kg^{-1} butorphanol [20 mg mL^{-1}; Kyron Laboratories (Pty) Ltd, South Africa], 0.8 mg kg^{-1} nalbuphine (20 mg mL^{-1}; Vtech Pty Ltd, South Africa) or 2 mL sterile water [Water for Injection; Kyron Laboratories (Pty) Ltd] was injected. These doses were established in a pilot dose–response study, in which incremental doses were administered to etorphine-immobilized goats

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

until a maximal improvement in peripheral haemoglobin oxygen saturation, using pulse oximetry (Nonin 9847V; Nonin Medical, MN, USA), was attained. Naltrexone (2 mg kg^{-1}; Trexonil 50 mg kg^{-1}; Wildlife Pharmaceuticals Pty Ltd) was administered intravenously (IV) 28 minutes after the etorphine injection.

Experimental procedures

The experiment consisted of three trials in which each goat was given etorphine + sterile water (control), etorphine + nalbuphine, and etorphine + butorphanol, respectively, in random order at 2 week intervals. Trial randomization was determined using a computer-generated number sequence. Each goat was weighed before each trial and then placed on a table in sternal recumbency with the head and neck kept straight by a handler who held the horns to ensure optimal airway patency.

A 22 gauge IV catheter (Introcan; B Braun Melsungen AG, Germany) was placed in one of the auricular arteries and connected to a three-way stopcock valve [Sabex Manufacturing (Pty) Ltd, South Africa]. A second 22 gauge catheter was placed in an auricular vein in the opposite ear to facilitate IV drug administration. A canine anaesthetic face mask (J-298C; Jorgensen Laboratories, CO, USA) was placed over the nose and mouth of the goat in a way that minimized additional dead space. A tight seal between the goat's muzzle and the face mask was achieved by using a modified latex glove. The face mask was connected to a two-way valve (2730; Hans Rudolph, Inc., OK, USA), which directed all expired air into the PowerLab Exercise Physiology System (ML870B80; ADInstruments Pty Ltd, NSW, Australia) via a respiratory flow head (MLT300L; ADInstruments Pty Ltd) linked to a spirometer (ML141; ADInstruments Pty Ltd) and a gas mixing chamber (MLA245; ADInstruments Pty Ltd), in which expired gas temperature was measured by a thermistor pod (ML309; ADInstruments Pty Ltd). The data from these modules were collected via the PowerLab 8/30 amplifier (ML870; ADInstruments Pty Ltd) and integrated with the Metabolic Module software (ADInstruments Pty Ltd) to measure or calculate minute ventilation, tidal volume, f_R, $\dot{V}O_2$ and $\dot{V}CO_2$. Before each set of measurements, the spirometer was zeroed and calibrated using a 3 L calibration syringe.

Data were recorded for 3 minutes before the injection of etorphine and throughout the immobilization. Heparinized 0.8 mL arterial blood samples were drawn 2 minutes before etorphine injection and at 5, 10, 15, 20 and 25 minutes thereafter. PaO_2 and $PaCO_2$ were measured immediately after sample collection using a pre-calibrated blood gas analyser with pre-calibrated blood gas cassettes [Roche OPTI CCA Analyser + OPTI cassette B; Kat Medical (Pty) Ltd, South Africa]. Partial pressures were measured and reported at 37 °C. Body temperature was measured with a thermal couple probe inserted into the rectum and connected to a thermometer (BAT-12; Physitemp Instruments, Inc., NJ, USA).

The quality of immobilization was scored at 1 minute after each arterial blood sample. Etorphine induces immobilization rather than complete anaesthesia; hence, we used an immobilization score rather than a standardized evaluation of the depth of anaesthesia to assess the level of immobilization. Scores ranged from 1 (fully awake) to 5 (deep immobilization). A score of 2 indicated a light plane of immobilization with movement of one or more body parts, and scores of 3 and 4 indicated appropriate immobilization with and without, respectively, the occasional movement of one body part.

Data analysis

We used GraphPad Prism Version 6.0 for Mac OS X (GraphPad Software, Inc., CA, USA) for statistical analyses. All results are reported as the mean ± SD. A p-value of < 0.05 was considered to indicate statistical significance. For all variables measured by the blood gas analyser and PowerLab Exercise Physiology System, two-way repeated-measures analyses of variance (ANOVAs) followed by Tukey's multiple comparisons tests were used to test for differences between responses to nalbuphine, butorphanol and sterile water (control) at −2, 5, 10, 15, 20 and 25 minutes, and between time-points for each intervention. We compared changes from baseline, rather than absolute values, to account for variability in baseline values.

Results

The goats' rectal temperatures ranged from 38.0 °C to 39.4 °C throughout the trials; this range is within the normal range for laboratory goats (Goodwin 1998). There was no difference in the goats' temperatures between the trials ($F_{(2,21)} = 0.12$, $p = 0.89$).

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

Immobilization

Although the exact time to immobilization varied among individuals, all goats were completely immobile (absent palpebral and ear flick reflexes; no movement of any body parts) at 5 minutes after etorphine administration (median immobilization score of 4 across all trials). Immobilization was maintained throughout the experimental procedure in the control trial in all goats (Table 1). Both nalbuphine and butorphanol administration resulted in a decrease in the level of immobilization. Median immobilization scores at 15 and 20 minutes were 2.5 and 2.0 following nalbuphine and butorphanol, respectively.

Changes in f_R

Etorphine led to a decrease in f_R at 5 minutes post-administration ($F_{(5,105)} = 32.96$, $p < 0.0001$) and nalbuphine administration did not correct this hypopnea (Fig. 1a & Table 2). By contrast, after butorphanol administration f_R values increased ($p < 0.05$), were similar to baseline values ($p > 0.05$) and were greater than those in the control group at all time-points ($p < 0.01$).

Table 1 Immobilization scores at 5 minute intervals in goats receiving etorphine (0.1 mg kg^{-1}) intramuscularly and one of nalbuphine (0.8 mg kg^{-1}), butorphanol (0.1 mg kg^{-1}) or sterile water intravenously 8 minutes later

	Immobilization score				
	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes
Goat 1					
Control	4	3	4	4	4
Butorphanol	3	2	2	2	2
Nalbuphine	3	2	2	2	2
Goat 2					
Control	3	4	3	3	2
Butorphanol	3	2	2	2	2
Nalbuphine	3	2	2	2	2
Goat 3					
Control	4	4	4	4	4
Butorphanol	3	2	2	2	2
Nalbuphine	3	2	3	3	3
Goat 4					
Control	3	4	4	4	4
Butorphanol	4	3	2	2	2
Nalbuphine	4	3	2	2	2
Goat 5					
Control	4	4	3	4	3
Butorphanol	4	2	2	2	2
Nalbuphine	4	2	2	2	2
Goat 6					
Control	4	4	3	3	3
Butorphanol	4	2	2	2	2
Nalbuphine	4	2	3	3	2
Goat 7					
Control	4	4	4	4	4
Butorphanol	4	1	1	1	1
Nalbuphine	4	3	3	3	2
Goat 8					
Control	4	4	4	4	4
Butorphanol	4	3	2	2	2
Nalbuphine	4	4	4	4	4

The score was noted by the same veterinarian in all trials on a scale of 1–5, where 1 = fully awake; 2 = light immobilization with movement of one or more body parts; 3 = appropriate immobilization with occasional movement of one body part; 4 = appropriate, complete immobilization; 5 = deep immobilization.

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

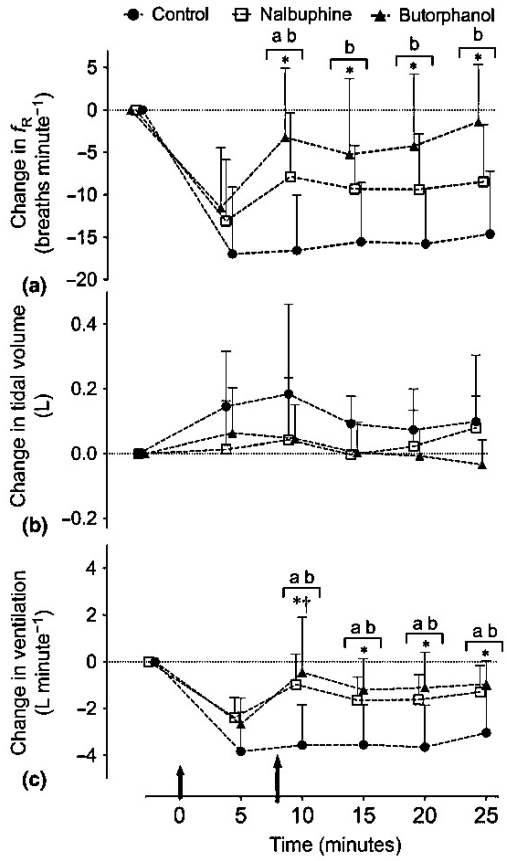


Figure 1 Effects of intravenous nalbuphine, butorphanol and sterile water (control) administered to eight goats at 8 minutes (grey arrow) after injection of intramuscular etorphine (black arrow, time 0) on changes in (a) respiratory rate (f_R), (b) tidal volume and (c) ventilation. * and † indicate differences from pre-administration values (5 minutes) in the butorphanol and nalbuphine trials, respectively. Differences between treatment trials are indicated by the letters a (nalbuphine *versus* control) and b (butorphanol *versus* control) (repeated-measures two-way ANOVA).

Changes in tidal volume

There were no significant changes in tidal volume over the duration of the immobilization ($F_{(5,105)} = 2.1$, $p = 0.07$) or between treatments ($F_{(2,21)} = 2.1$, $p = 0.14$) (Fig. 1b & Table 2).

Changes in ventilation

Ventilation changed over time ($F_{(5,105)} = 29.5$, $p < 0.0001$) and between treatments ($F_{(2,21)} = 6.9$, $p = 0.005$). The decrease in ventilation following etorphine administration was similar across all groups (Fig. 1c & Table 2). In the control trial, the ventilation of the goats remained below baseline levels throughout the immobilization period. Nalbuphine administration led to an increase in ventilation at 10 minutes ($p < 0.05$), but at 15, 20 and 25 minutes ventilation was lower than before etorphine administration ($p < 0.05$) and no different from that at 5 minutes. However, nalbuphine led to improved ventilation compared with the control group ($p < 0.05$). Butorphanol administration corrected the etorphine-induced hypoventilation and values at 10, 15, 20 and 25 minutes were greater than in the control group ($p < 0.05$) and no different from baseline values.

Changes in arterial blood gases

During the course of the immobilization, PaO_2 changed over time in all trials ($F_{(5,105)} = 58.93$, $p < 0.0001$). Five minutes after etorphine administration, PaO_2 decreased in all groups. In the control trial PaO_2 gradually improved and was higher at 15 minutes ($p < 0.05$), 20 minutes ($p < 0.01$) and 25 minutes ($p < 0.001$) compared with at 5 minutes post-etorphine administration. Despite this gradual improvement, the goats remained hypoxaemic throughout the immobilization period with PaO_2 lower ($p < 0.001$) than pre-etorphine values at all time-points. Nalbuphine and butorphanol increased PaO_2 significantly compared with that in the control group. This improvement was rapid and corrected the hypoxaemia; values were similar to those at baseline throughout the immobilization period in the butorphanol trial and at 10 and 25 minutes in the nalbuphine trial (Fig. 2a & Table 2).

Five minutes after etorphine administration, $PaCO_2$ increased in all groups ($F_{(5,105)} = 46$, $p < 0.0001$). In the control trial, $PaCO_2$ remained elevated throughout the immobilization period. Nalbuphine and butorphanol administration led to decreases in $PaCO_2$ at 10, 15, 20 and 25 minutes compared with at 5 minutes, but did not achieve pre-etorphine values (Fig. 2b & Table 2).

Changes in $\dot{V}O_2$ and $\dot{V}CO_2$

Oxygen consumption in the goats did not differ between treatment interventions at any time-point ($F_{(2,21)} = 0.78$, $p = 0.47$), but within the nalbuphine and butorphanol trials, $\dot{V}O_2$ changed over time ($F_{(5,105)} = 11.23$, $p < 0.0001$). In the control

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

Table 2 Respiratory parameters in goats receiving etorphine (0.1 mg kg^{-1}) intramuscularly and one of nalbuphine (0.8 mg kg^{-1}), butorphanol (0.1 mg kg^{-1}) or sterile water intravenously 8 minutes later

	Baseline	5 minutes	10 minutes	15 minutes	20 minutes	25 minutes
f_R (breaths $minute^{-1}$)						
Control	30 ± 7	13 ± 5	13 ± 5	14 ± 1	14 ± 2	15 ± 3
Butorphanol	24 ± 8	13 ± 4	21 ± 7*,†	19 ± 4†	20 ± 5†	23 ± 6*,†
Nalbuphine	23 ± 8*	10 ± 3	16 ± 6	14 ± 4	14 ± 4	15 ± 5
Tidal volume (L)						
Control	0.35 ± 0.17	0.49 ± 0.21†	0.53 ± 0.27	0.44 ± 0.20†	0.42 ± 0.21†	0.45 ± 0.19†
Butorphanol	0.32 ± 0.12	0.38 ± 0.19†	0.36 ± 0.20†	0.32 ± 0.13†	0.31 ± 0.09†	0.29 ± 0.12†
Nalbuphine	0.25 ± 0.11	0.26 ± 0.09*,†	0.29 ± 0.15*,†	0.25 ± 0.06†	0.27 ± 0.15†	0.33 ± 0.22†
Ventilation (L $minute^{-1}$)						
Control	9.82 ± 4.62	5.98 ± 3.14	6.25 ± 3.10	6.27 ± 2.96	6.16 ± 2.92	6.78 ± 3.30
Butorphanol	7.36 ± 3.75	4.69 ± 3.04	6.91 ± 4.42†	6.15 ± 3.01†	6.25 ± 2.79†	6.40 ± 3.27†
Nalbuphine	5.09 ± 1.01*	2.70 ± 0.62	4.11 ± 1.32†	3.44 ± 0.99	3.46 ± 1.01	3.79 ± 0.76
PaO_2 (mmHg) (kPa)						
Control	67 ± 3 (8.8 ± 0.4)	45 ± 7 (6.0 ± 0.9)†	51 ± 5 (6.8 ± 0.7)†	52 ± 4 (6.9 ± 0.5)†	54 ± 5 (7.2 ± 0.7)†	55 ± 5 (7.3 ± 0.7)†
Butorphanol	67 ± 2 (8.8 ± 0.3)	44 ± 6 (5.9 ± 0.8)	63 ± 11 (8.4 ± 1.5)*,†	61 ± 4 (8.1 ± 0.5)*,†	60 ± 6 (8.0 ± 0.8)†	61 ± 6 (8.1 ± 0.8)†
Nalbuphine	67 ± 4 (8.8 ± 0.5)	44 ± 6 (5.9 ± 0.8)	63 ± 10 (8.4 ± 1.3)*,†	57 ± 7 (7.6 ± 0.9)	57 ± 7 (7.6 ± 0.9)	62 ± 6 (8.3 ± 0.8)†
$PaCO_2$ (mmHg) (kPa)						
Control	33 ± 2 (4.4 ± 0.3)	44 ± 4 (5.9 ± 0.5	44 ± 4 (5.9 ± 0.5)	45 ± 3 (6.0 ± 0.4)	45 ± 3 (6.0 ± 0.4)	44 ± 4 (5.9 ± 0.5)
Butorphanol	34 ± 2 (4.5 ± 0.3)	46 ± 3 (6.1 ± 0.4)	39 ± 7 (5.2 ± 0.9)	39 ± 3 (5.2 ± 0.4)*	39 ± 5 (5.2 ± 0.7)*	39 ± 5 (5.2 ± 0.6)*
Nalbuphine	34 ± 3 (4.5 ± 0.4)	45 ± 4 (6.0 ± 0.5)	40 ± 7 (5.3 ± 0.9)	41 ± 6 (5.5 ± 0.8)	41 ± 6 (5.5 ± 0.8)	38 ± 6 (5.1 ± 0.8)*
$\dot{V}O_2$ (L $minute^{-1}$)						
Control	0.15 ± 0.06	0.16 ± 0.08†	0.19 ± 0.10†	0.19 ± 0.09†	0.18 ± 0.08†	0.18 ± 0.09†
Butorphanol	0.13 ± 0.09	0.14 ± 0.10†	0.21 ± 0.10	0.14 ± 0.07†	0.16 ± 0.07†	0.15 ± 0.07†
Nalbuphine	0.10 ± 0.03	0.08 ± 0.02*,†	0.14 ± 0.05	0.10 ± 0.02*,†	0.10 ± 0.02†	0.10 ± 0.01*,†
$\dot{V}CO_2$ (L $minute^{-1}$)						
Control	0.17 ± 0.08	0.12 ± 0.07	0.13 ± 0.08	0.14 ± 0.07	0.14 ± 0.07	0.15 ± 0.08†
Butorphanol	0.13 ± 0.08	0.11 ± 0.07†	0.17 ± 0.10	0.14 ± 0.06†	0.14 ± 0.06†	0.14 ± 0.06†
Nalbuphine	0.10 ± 0.03	0.07 ± 0.02	0.11 ± 0.04†	0.09 ± 0.02†	0.09 ± 0.03†	0.09 ± 0.02†

PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide; f_R, respiratory rate; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production. *Values differ significantly from those of the control trial ($p < 0.05$). †Values do not differ from baseline values for each trial ($p \geq 0.05$).

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

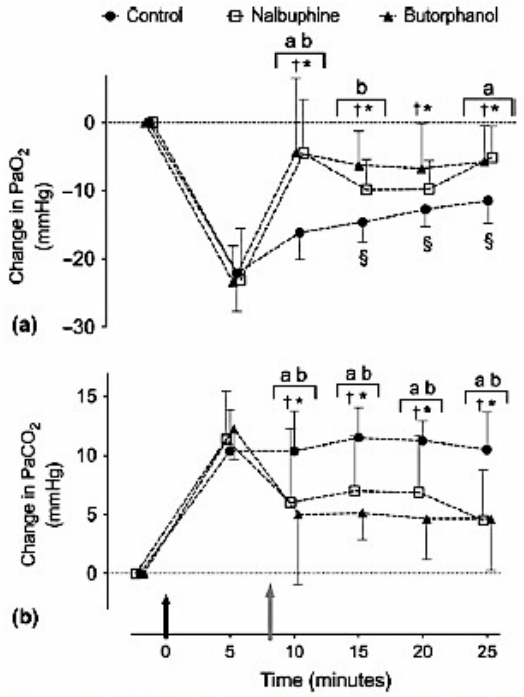


Figure 2 Effects of intravenous nalbuphine, butorphanol and sterile water (control) administered to eight goats at 8 minutes (grey arrow) after injection of intramuscular etorphine (black arrow, time 0) on changes in (a) partial pressure of arterial oxygen (PaO_2) and (b) partial pressure of arterial carbon dioxide ($PaCO_2$). The symbols *, † and § indicate differences from pre-administration values (5 minutes) in the butorphanol, nalbuphine and control trials, respectively. Differences between treatment trials are indicated by the letters a (nalbuphine *versus* control) and b (butorphanol *versus* control) (repeated-measures two-way ANOVA).

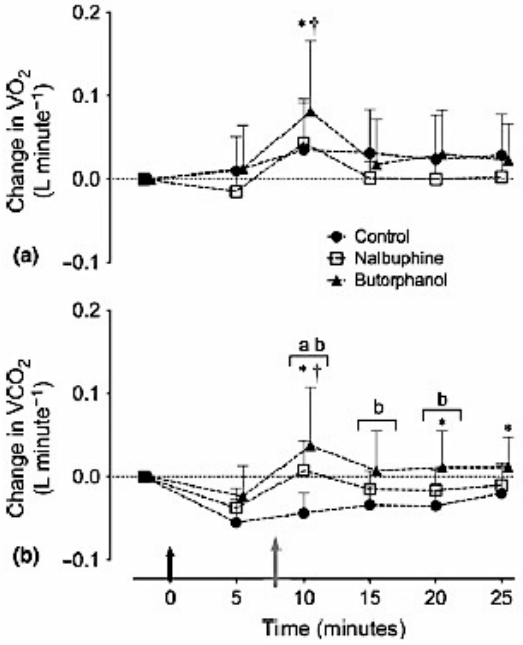


Figure 3 Effects of intravenous nalbuphine, butorphanol and sterile water (control) administered to eight goats at 8 minutes (grey arrow) after injection of intramuscular etorphine (black arrow, time 0) on changes in (a) oxygen consumption ($\dot{V}O_2$) and (b) carbon dioxide production ($\dot{V}CO_2$). * and † indicate differences from pre-administration values (5 minutes) in the butorphanol, nalbuphine and control trials, respectively. Differences between treatment trials are indicated by the letters a (nalbuphine *versus* control) and b (butorphanol *versus* control) (repeated-measures two-way ANOVA).

group, $\dot{V}O_2$ remained constant throughout the trial. At 10 minutes following nalbuphine administration, $\dot{V}O_2$ increased above baseline values ($p < 0.001$), but then decreased to pre-etorphine values at 15, 20 and 25 minutes. Similarly, butorphanol administration led to an increase in $\dot{V}O_2$ at 10 minutes ($p < 0.0001$), followed by a decrease to pre-etorphine values at 15, 20 and 25 minutes (Fig. 3a & Table 2).

The change in $\dot{V}CO_2$ following treatment interventions differed among trials ($F_{(2,21)} = 4.65$, $p = 0.02$) and time-points ($F_{(5,105)} = 10.53$, $p < 0.0001$). Butorphanol and nalbuphine both caused an increase in $\dot{V}CO_2$ in etorphine-immobilized goats. At 10 minutes, $\dot{V}CO_2$ was increased above pre-etorphine values after administration of both nalbuphine ($p < 0.01$) and butorphanol ($p < 0.0001$). By 25 minutes, $\dot{V}CO_2$ in the goats had returned to pre-etorphine values in all trials (Fig. 3b & Table 2).

Discussion

Nalbuphine and butorphanol improved respiratory parameters in etorphine-immobilized goats compared with those in goats to which no opioid agonist-antagonist was administered, but butorphanol elicited greater improvement than nalbuphine. The goats' respiratory frequency and, consequently, ventilation were corrected to pre-etorphine values after butorphanol, but not after nalbuphine administration. However, both nalbuphine and butorphanol restored PaO_2 to pre-etorphine values. Conversely, the immobilized goats retained elevated $PaCO_2$ levels following both nalbuphine and butorphanol. Although nalbuphine improved and butorphanol corrected most

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

respiratory parameters, both opioid agonist-antagonists also caused increased arousal in the immobilized goats, with butorphanol causing greater arousal than nalbuphine. The increases in $\dot{V}O_2$ and $\dot{V}CO_2$ immediately after the administration of butorphanol and nalbuphine indicated that metabolism increased as the level of immobilization decreased.

Etorphine is rarely used for the immobilization of wildlife without additional sedatives or tranquillizers and at the high dose of 0.1 mg kg^{-1} used in this study. We chose to immobilize the goats with pure etorphine in order to accurately assess the interactions of different opioid drugs, without the confounding effects of sedatives or tranquillizers. Although goats provide a valuable model for assessing pharmacodynamic responses of drugs in etorphine-immobilized ungulates, the model is limited by the fact that responses may differ in certain wildlife species, and under different conditions, given the complexity of opioid pharmacology both within and between species. However, controlled laboratory studies ensure that the minimum number of animals can be used to provide valuable results. Ultimately, promising results obtained in a laboratory setting should be verified in the target species. Another benefit of a controlled, randomized, crossover laboratory study such as ours is that it can reduce bias, which may not be always possible in a field setting, especially when scientific trials are combined with routine wildlife management procedures (in order to reduce unnecessary immobilizations of wild animals).

Butorphanol is widely used by wildlife veterinarians because it is readily available and believed to antagonize the μ-opioid receptor respiratory depressant effects of etorphine, without causing unwanted arousal. However, in non-human primates, butorphanol has been characterized as a low-efficacy μ-receptor agonist, rather than as a μ-antagonist (Butelman et al. 1995; Vivian et al. 1999). Recent studies in white rhinoceros have also shown that the beneficial effects of butorphanol on respiratory parameters are related to a decrease in immobilization level more than they are to antagonism of μ-opioid-induced hypoventilation (Miller et al. 2013; Haw et al. 2014). We therefore compared butorphanol with nalbuphine because results from studies in mice (Schmidt et al. 1985) and humans (Bowdle et al. 1987) indicated that nalbuphine may be a more potent μ-antagonist than butorphanol. Indeed, in humans anaesthetized with nitrous oxide, butorphanol caused significantly greater respiratory depression than nalbuphine when administered at equianalgesic doses (Zucker et al. 1987). Our findings in etorphine-immobilized goats appear to contradict the results of these earlier studies because they suggest that butorphanol has more potent μ-opioid antagonistic effects than does nalbuphine. Therefore, it appears that the activity of butorphanol and nalbuphine at different opioid receptors are not consistent across species.

In our study, $PaCO_2$ did not improve as much as the corresponding minute ventilation following either nalbuphine or butorphanol administration. Similarly, in humans under fentanyl anaesthesia, $PaCO_2$ did not improve as much as minute ventilation after butorphanol (Bowdle et al. 1987). That $PaCO_2$ changed to a lesser extent than did the corresponding minute ventilation indicates that either $\dot{V}CO_2$ increased substantially after butorphanol and nalbuphine, or dead space ventilation increased out of proportion to alveolar ventilation. Our results show that $\dot{V}CO_2$ did increase transiently after nalbuphine, with a more sustained increase after butorphanol administration. A disproportionate increase in dead space ventilation may also have been an important factor, especially as the increased minute ventilation resulted from an increased f_R, rather than tidal volume.

In parallel with $\dot{V}CO_2$, $\dot{V}O_2$ also increased, indicating a rise in metabolism after the administration of butorphanol and nalbuphine. This increase in metabolism corresponded with an increase in movement and a decrease in immobilization level. Our study therefore supports the current hypothesis that the desirable effects of μ-opioid receptor activation, such as analgesia and catatonia, cannot be completely separated from the adverse effects, such as respiratory depression (Kieffer 1999). Earlier reports suggest different μ-receptor subtypes for the analgesic and respiratory depressant effects of μ-agonists (Ling et al. 1985). However, molecular studies have now confirmed that only a single μ-receptor gene exists (Pasternak & Pan 2011). The variability among species and individual responses to opioids may result from extensive alternative splicing of the μ-opioid receptor such that a number of splice variants, which lack certain amino acid sequences, are generated (Pasternak 2014). The number of splice variants and the exact amino acid sequencing of the variants differ among species (Pasternak 2014). It was recently discovered that opioid receptors have a crystal structure, which indicates that these receptors are dynamic systems and that

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

ligands may affect the conformational properties of the receptor, leading to the preferential stimulation of certain intracellular pathways over others (Shang & Filizola 2015). Developing opioid ligands that bias a specific intracellular pathway, rather than a whole receptor, may provide a more effective route to developing immobilizing and analgesic agents with reduced side effects (Shang & Filizola 2015).

The present study has confirmed that opioid agonist-antagonists perform differently in etorphine-immobilized herbivores, compared with primates and rodents. Although nalbuphine has been reported to offer advantages over butorphanol (Pallasch & Gill 1985), we found no such advantages in our ungulate model. Indeed, butorphanol may be a better agonist-antagonist because it reversed the etorphine-induced respiratory depression more efficiently than nalbuphine. As a result of the complexity of opioid receptor pharmacology, and differences in responses to opioid ligands within and between species, it may be impossible to find an opioid agonist-antagonist that uniformly targets opioid receptors in a beneficial manner in respiration-compromised wildlife of all species. Therefore, in the future, we should seek to find alternative pharmacological interventions that reverse opioid-induced respiratory depression without affecting the intended use of the opioid agonists, such as analgesia or the level of immobilization. There are agents that have been shown to reverse opioid-induced respiratory depression through actions on non-opioid receptors such as ampakines (Oertel et al. 2010), serotonergic agonists (Meyer et al. 2006) and potassium channel blockers (Roozekrans et al. 2014). These novel non-opioid 'respiratory stimulants' should be evaluated in more detail in wildlife. In the meantime, butorphanol and nalbuphine can be used to improve respiratory parameters in immobilized wildlife, with butorphanol being more effective, but unwanted arousal can occur with both.

Acknowledgements

The authors thank the staff of the Central Animal Services of the University of the Witwatersrand for their animal management and technical support, and the staff and students of the Brain Function Research Group for their help with animal handling and data collection. This work was funded by a Faculty Research Committee Grant, University of the Witwatersrand, awarded to AJH and a Thuthuka grant from the National Research Foundation, South Africa, awarded to LCRM.

Authors' contributions

AJH contributed to the conception of the study, and the acquisition, analysis and interpretation of data, and drafted the first manuscript. LCRM and AF contributed to the conception of the study and the interpretation of data, and to the critical revision of the manuscript for intellectual content.

References

Bowdle TA (1998) Adverse effects of opioid agonists and agonist-antagonists in anaesthesia. Drug Saf 19, 173–189.

Bowdle TA, Greichen SL, Bjurstrom RL et al. (1987) Butorphanol improves CO_2 response and ventilation after fentanyl anesthesia. Anesth Analg 66, 517–522.

Burroughs R, Meltzer D, Morkel P (2012) Applied pharmacology. In: Chemical and Physical Restraint of Wild Animals (2nd edn). Kock MD, Meltzer D, Burroughs R (eds). IWVS Africa, South Africa. pp. 53–62.

Butelman ER, Winger G, Zernig G et al. (1995) Butorphanol: characterization of agonist and antagonist effects in rhesus monkeys. J Pharmacol Exp Ther 272, 845–853.

Dahan A, Aarts L, Smith T (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. Anesthesiology 112, 226–238.

Goodwin SD (1998) Comparison of body temperatures of goats, horses, and sheep measured with a tympanic infrared thermometer, an implantable microchip transponder, and a rectal thermometer. J Am Assoc Lab Anim Sci 37, 51–55.

Haw A, Hofmeyr M, Fuller A et al. (2014) Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*). BMC Vet Res 10, 253.

Hoskin PJ, Hanks GW (1991) Opioid agonist-antagonist drugs in acute and chronic pain states. Drugs 41, 326–344.

Kieffer BL (1999) Opioids: first lessons from knockout mice. Trends Pharmacol Sci 20, 19–26.

Kilkenny C, Browne WJ, Cuthill IC et al. (2010) Improving bioscience research reporting: the ARRIVE Guidelines for Reporting Animal Research. PLoS Biol 8, e1000412.

Ling G, Spiegel K, Lockhart S et al. (1985) Separation of opioid analgesia from respiratory depression: evidence for different receptor mechanisms. J Pharmacol Exp Ther 232, 149–155.

Meyer LCR, Fuller D, Mitchell D (2006) Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats.

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

Am J Physiol Regul Integr Comp Physiol 290, R405–R413.

Miller M, Buss P, Joubert J et al. (2013) Use of butorphanol during immobilization of free-ranging white rhinoceros (*Ceratotherium simum*). J Zoo Wildl Med 44, 55–61.

Oertel BG, Felden L, Tran PV et al. (2010) Selective antagonism of opioid-induced ventilatory depression by an ampakine molecule in humans without loss of opioid analgesia. Clin Pharmacol Ther 87, 204–211.

Pallasch TJ, Gill CJ (1985) Butorphanol and nalbuphine: a pharmacologic comparison. Oral Surg Oral Med Oral Pathol 59, 15–20.

Pasternak G (2014) Opioids and their receptors: are we there yet? Neuropharmacology 76, 198–203.

Pasternak G, Pan Y (2011) Mu opioid receptors in pain management. Acta Anaesthesiol Taiwan 49, 21–25.

Roozekrans M, van der Schrier R, Okkerse P et al. (2014) Two studies on reversal of opioid-induced respiratory depression by BK-channel blocker GAL021 in human volunteers. Anesthesiology 121, 459–468.

Schmidt WK, Tam SW, Schotzberger GS et al. (1985) Nalbuphine. Drug Alcohol Depend 14, 339–362.

Shang Y, Filizola M (2015) Opioid receptors: structural and mechanistic insights into pharmacology and signaling. Eur J Pharmacol 763, 206–213.

Vivian JA, DeYoung MB, Sumpter TL et al. (1999) κ-opioid receptor effects of butorphanol in rhesus monkeys. J Pharmacol Exp Ther 290, 259–265.

Zucker JR, Neuenfeldt T, Freund PR (1987) Respiratory effects of nalbuphine and butorphanol in anesthetized patients. Anesth Analg 66, 879–881.

Received 5 August 2015; accepted 11 December 2015.

© 2016 Association of Veterinary Anaesthetists and the American College of Veterinary Anesthesia and Analgesia

CHAPTER 3

Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*)

Haw A, Hofmeyr M, Fuller A, Buss P, Miller M, Fleming G and Meyer L

***BMC Veterinary Research* 10:253, 2014**

Haw et al. BMC Veterinary Research 2014, 10:253
http://www.biomedcentral.com/1746-6148/10/253

BMC Veterinary Research

RESEARCH ARTICLE Open Access

Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*)

Anna Haw[1], Markus Hofmeyr[3,2], Andrea Fuller[1], Peter Buss[3,6], Michele Miller[4], Gregory Fleming[5] and Leith Meyer[1,2*]

Abstract

Background: Opioid-induced immobilization is associated with severe respiratory depression in the white rhinoceros. We evaluated the efficacy of butorphanol and oxygen insufflation in alleviating opioid-induced respiratory depression in eight boma-managed rhinoceros.

Results: Chemical immobilization with etorphine, azaperone and hyaluronidase, as per standard procedure for the white rhinoceros, caused severe respiratory depression with hypoxaemia ($P_aO_2 = 27 \pm 7$ mmHg [mean ± SD]), hypercapnia ($P_aCO_2 = 82 \pm 6$ mmHg) and acidosis (pH = 7.26 ± 0.02) in the control trial at 5 min. Compared to pre-intervention values, butorphanol administration (without oxygen) improved the P_aO_2 (60 ± 3 mmHg, $F_{(3,21)} = 151.9$, $p < 0.001$), P_aCO_2 (67 ± 4 mmHg, $F_{(3,21)} = 22.57$, $p < 0.001$) and pH (7.31 ± 0.06, $F_{(3,21)} = 27.60$, $p < 0.001$), while oxygen insufflation alone exacerbated the hypercapnia (123 ± 20 mmHg, $F_{(3,21)} = 50.13$, $p < 0.001$) and acidosis (7.12 ± 0.07, $F_{(3,21)} = 110.6$, $p < 0.001$). Surprisingly, butorphanol combined with oxygen fully corrected the opioid-induced hypoxaemia ($P_aO_2 = 155 \pm 53$ mmHg) and reduced the hypercapnia over the whole immobilization period ($p < 0.05$, areas under the curves) compared to the control trial. However, this intervention (butorphanol + oxygen) did not have any effect on the arterial pH.

Conclusions: Oxygen insufflation combined with a single intravenous dose of butorphanol improved the immobilization quality of boma-managed white rhinoceros by correcting the opioid-induced hypoxaemia, but did not completely reverse all components of respiratory depression. The efficacy of this intervention in reducing respiratory depression in field-captured animals remains to be determined.

Keywords: Anaesthesia, Hypoxia, Hypercapnia, Acidaemia, Blood gases, Opioids, Partial-opioid antagonist

Background

Potent opioids, such as etorphine hydrochloride, are used routinely for the chemical immobilization of white rhinoceros (*Ceratotherium simum*), an essential procedure for the management and conservation of this species. Whilst these potent mu-agonist opioids induce a rapid immobilization, respiratory depression characterized by hypoventilation, hypoxaemia, hypercapnia and acidaemia is common [1-6]. The opioid-induced respiratory depression also is compounded by rigidity of the animal's thoracic musculature, which impairs respiratory excursions [4]. Respiratory depression is arguably the most significant complication in the immobilization and anaesthesia of white rhinoceros [1,7,8], with animals shown to have an arterial oxygen partial pressure of less than 40 mmHg [2].

In an effort to reduce the respiratory depression, attempts have been made to selectively antagonize the undesirable side-effects of pure-opioid agonists while preserving their potent analgesic, sedative and catatonic effects, using mixed opioid agonists-antagonists such as nalorphine [7] and butorphanol [5,9]. Nalorphine is no longer readily available,

* Correspondence: Leith.Meyer@up.ac.za
[1]Brain Function Research Group, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
[2]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa
Full list of author information is available at the end of the article

BioMed Central

© 2014 Haw et al.; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

so butorphanol has become widely used in the field [8]. However, whether butorphanol effectively reduces respiratory depression is not clear from the few studies that have been done [5,9]. Oxygen administered intranasally [10] or via nasotracheal intubation [3] also has been used to increase blood oxygen levels in etorphine-immobilized white rhinoceros, but field practitioners have questioned its efficacy and safety (Buss, personal communication).

In this paper, we critically evaluate combinations of butorphanol and oxygen insufflation in a controlled boma (small enclosure used to temporarily hold wild animals) setting to determine their efficacy in alleviating opioid-induced respiratory depression in white rhinoceros.

Methods

This project was approved by the Animal Use and Care Committee, South African National Parks (SANParks) as well as the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance 2012/23/04).

Study area and sample population

Eight sub-adult (3–5 year old) male white rhinoceros were captured within Kruger National Park (−24.98984 S, 31.59263 E, Alt. 317 m) and housed individually in holding facilities (bomas) according to SANParks' standard operating procedures. Food intake (lucerne and hay), defaecation and general demeanour were assessed daily for each rhinoceros. The trial started after a one month adaptation period. Data collection occurred from August to December 2012 and all immobilizations took place between 05H20 and 10H50. Black globe temperature (thermal load) ranged from 10°C to 43°C and barometric pressure varied from 737.0 to 745.9 mmHg.

Chemical immobilization and experimental design

At each trial, the rhinoceros was immobilized with a combination of 2–3 mg etorphine hydrochloride (M99®, Novartis, Kempton Park, South Africa, 9.8 mg/ml), 30–45 mg azaperone (Stressnil®, Janssen Pharmaceutical Ltd., Halfway House, South Africa, 40 mg/ml), and 2500 i.u. hyaluronidase (lyophilized hyalase, Kyron Laboratories, South Africa). Drug doses were determined by body mass (Buss, personal communication, Table 1). Immobilizing drugs were administered into the nuchal hump using a 3 ml plastic dart with a 60 mm needle, fired with a CO_2-powered dart-gun (Dan-Inject, South Africa). Once safe to handle the rhinoceros was blindfolded and positioned in lateral recumbency (Time 0) with padding under the head to ensure the lower nostril remained patent. Trials were performed only if the animal became immobile within 15 minutes of darting. Recumbency position was altered between left and right body side at each immobilization.

The experiment consisted of four trials in which the interventions butorphanol (15 mg per mg etorphine, Kyron Laboratories, Benrose, South Africa, 20 mg/ml), butorphanol (15 mg per mg etorphine) + oxygen insufflation (30 L/min), oxygen insufflation (30 L/min) on its own, and sterile water (control) were evaluated and compared as measures to support the respiratory physiology of the immobilized white rhinoceros. Each rhinoceros received each intervention for the four trials in a randomised order at two-week intervals. The interventions were administered 6 min after the rhinoceros became laterally recumbent. Clinical data and samples were collected at 5 min after animals became laterally recumbent (before the intervention) and every 5 minutes thereafter for a 20-minute immobilization period. In the two trials where butorphanol was not administered at 6 min (oxygen only and control interventions), butorphanol (15 mg per mg etorphine) was administered at 21 minutes to facilitate arousal and loading into a crate.

Table 1 Drug doses for white rhinoceros immobilization (Buss)

Weight (kg)	Etorphine (mg)	Azaperone (mg)	Butorphanol (mg)	Naltrexone (mg)
500-750	1.5	22.5	22.5	30
750-1000	2.0	30.0	30.0	40
1000-1250	2.5	37.5	37.5	50
1250-1500	3.0	45.0	45.0	60
1500-1750	3.5	52.5	52.5	70
1750-2000	4.0	60.0	60.0	80

Clinical monitoring and data collection

Respiratory rate was monitored by an observer counting thoracic and abdominal excursions and feeling for expired air at the nares. The level of immobilization was assessed by observing body and ear movements at 5, 10, 15 and 20 min. The level of immobilization score ranged from 1 (no immobilizing or sedative effect) to 6 (excessive immobilization depth with respiration <3 breaths/min). Level 3 indicated a safe standing sedation, while levels 4 and 5 indicated recumbent immobilization with or without ear movement, respectively.

Arterial blood samples for blood gas analysis were collected from the medial auricular artery catheterized with a 22G × 1" IV catheter (Nipro Safelet Cath, Nipro Corporation) and secured in place with superglue (SuperGlue, Loctite®). A 0.5 ml sample was collected anaerobically into 1 ml heparinised syringes at 5, 10, 15 and 20 minutes after the rhinoceros became laterally recumbent. A final arterial sample was taken in the standing sedated rhinoceros, once positioned in a crate for weighing, 23 to 33 minutes after initial lateral recumbency. Arterial pH, partial pressures of carbon dioxide (P_aCO_2), and oxygen (P_aO_2), and haemoglobin oxygen saturation (S_aO_2, data not shown) were

measured immediately after blood sampling using a portable precalibrated blood gas analyzer with precalibrated blood gas cassettes (Roche OPTI CCA Analyzer + OPTI cassette B, Kat Medical, Johannesburg, South Africa). The blood gas analyzer could not measure S_aO_2 values below 60%. The catheter was maintained with a heparinised (Heparin 5000 i.u./ml; Fresenius, Port Elizabeth, South Africa) saline flush and 1–2 ml of blood was discarded before collection of each sample.

During the experiments in which oxygen was delivered, nasotracheal intubation was achieved using an equine stomach tube (9.5 mm od × 213 cm, Kyron Laboratories) as described by Bush and colleagues [3]. Tracheal intubation was verified by hearing the air pass through the tube during respiration. Oxygen was delivered at a constant flow rate of 30 L/min based on earlier reports [3,4] and a prior pilot study.

Twenty-one minutes into the recumbent period, the rhinoceros was stimulated to stand and was guided into a crate for weighing. The quality of arousal was assessed according to the amount of stimulation needed to get the animal into a standing position and ability to guide the animal into a crate using an interval scale from 1 to 5. One indicates that the animal gets up too quickly, is too awake and cannot be blindfolded. 2 and 3 indicate that the animal gets up and can be safely handled and walked, with minor (2) or moderate (3) stimulation. 4 indicates that excessive stimulation is needed to get the animal standing and walking, while 5 indicates that the animal struggles to get up even after excessive stimulation and is unable to walk.

Approximately 35 min after the animals became laterally recumbent, the effects of etorphine were reversed using naltrexone (50 mg/ml, Kyron Laboratories, South Africa) administered intravenously into an auricular vein at 20-times the etorphine dose. The animals were then released from the crate into their bomas. The quality of recovery was subjectively categorized as poor, average or good by an individual veterinarian.

All rhinoceros used in these trials were monitored using a standardized boma scoring system on a daily basis for changes in appetite, defaecation, and behaviour. In addition, haematological and biochemical analyses were performed at the time of each immobilization to assess any changes in health status.

Data analysis

Statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA). All results are expressed as means ± SD, and statistical differences with $p < 0.05$ were considered significant. For P_aO_2, P_aCO_2, pH and respiratory rate, a repeated measures two-way analysis of variance (ANOVA) followed by Bonferroni post-tests was used to test for differences between responses to butorphanol, butorphanol + oxygen, oxygen, and sterile water (control) at 5, 10, 15 and 20 min and between time points for each intervention. To determine the integrated response over time, areas under the response curves to butorphanol, butorphanol + oxygen, oxygen and sterile water (control) were calculated for these same variables (P_aO_2, P_aCO_2, pH and respiratory rate) for the period during lateral recumbency. A one-way ANOVA followed by a Student-Newman-Keuls (SNK) post hoc test was used to test for differences between these areas. The measurements taken while the animals were standing in the crate were analysed separately with a one-way ANOVA followed by a SNK post hoc test to compare differences between the trials in the standing rhinoceros.

Results

All rhinoceros were in good health and showed behaviour indicative of healthy boma-managed rhinoceros throughout the trial. Although, initially after being relocated to the boma, the animals lost weight, they gained body mass gradually over time (average gain of 16 kg during the final two-week interval). The initial weight loss, associated with a brief period of anorexia, normally occurs while the animals adapt to captivity. Results of serum biochemical and haematological analyses were considered within normal ranges for healthy white rhinoceros [11]; Miller, personal communication. Follow-up monitoring of other health parameters subsequent to the experiment indicated no change in health status.

Immobilization

The administration of etorphine (0.002 mg/kg), azaperone (0.03 mg/kg) and hyaluronidase (2500 i.u) led to rapid immobilization of rhinoceros (5 ± 1 minutes) after darting. There was no difference in time to recumbency between the four different trials (control, butorphanol, butorphanol + oxygen and oxygen) ($F_{(3,28)} = 0.95$, $p = 0.43$). The level of immobilization of the rhinoceros also did not differ significantly between different trials. When recumbent, the rhinoceros were usually relaxed with some ear movements (median immobilization level 4); occasionally they would enter a deeper state of immobilization where they would be fully relaxed without any ear movement (level 5).

Respiratory rate

Immobilization with etorphine, azaperone and hyaluronidase caused hypopnea (7 ± 2 breaths/min; normal mean [±SD] =19 [±0.6], breaths/min, [12]) which persisted throughout the immobilization period in animals that received the control (sterile water). There was no statistical difference ($F_{(3,28)} = 6.03$, $p > 0.05$) in mean respiratory rates between trials at t =5 min (butorphanol =6 ± 1; butorphanol + oxygen =7 ± 1;

oxygen =8 ± 3 breaths/min) (Figure 1A). Although respiratory rate in the butorphanol trial significantly ($F_{(3,21)}$ =48.30, p <0.001) increased following butorphanol administration to 10 breaths/min (SD 1) at t =10, this change was not different than mean respiratory rate for the control intervention (7 ± 2 breaths/min, t =10; p >0.05) (Figure 1A). However, when comparing the overall response over the immobilization period by comparing areas under the response curves (Figure 2A) respiratory rate did increase with butorphanol administration (p <0.05). Similarly, butorphanol combined with oxygen caused an initial increase in respiratory rate (9 ± 2 breaths per min at 10 min; $F_{(3,21)}$ =8.97, p <0.01). However, this improvement was not sustained and the respiratory rate over the full 20-min immobilization period was not different from the control trial (Figure 2A). Oxygen insufflation as sole support caused a decrease in respiratory rate at 10, 15 and 20 min, compared to 5 min ($F_{(3,21)}$ =37.62, p <0.001), and resulted in an overall lower respiratory rate than in the control trial (p <0.001, Figure 2A).

Partial pressure of arterial carbon dioxide (P_aCO_2)

The decrease in respiratory rate induced by chemical immobilization resulted in severe hypoventilation, as indicated by the extreme hypercapnia (82 ± 6 mmHg, normal =49 [±0.9] mmHg [12]) at 5 min in the control trial, which did not change over time ($F_{(3,21)}$ =2.68, p =0.073). The initial hypercapnia at 5 min was similar across rhinoceros in all trials (butorphanol =83 ± 9 mmHg; butorphanol + oxygen =79 ± 7 mmHg; oxygen =82 ± 8 mmHg) (Figure 1B). Butorphanol administration led to a decrease in P_aCO_2 from 5 to 10 min (67 ± 4 mmHg, p <0.001) which resulted in an overall improvement compared to the control (p <0.001, areas under the response curves, Figure 2B). The administration of butorphanol + oxygen did not result in an improvement in P_aCO_2 levels at 10, 15 or 20 min, compared to pre-intervention values ($F_{(3,21)}$ =1.31, p >0.05, P_aCO_2 = 72 ± 13 mmHg at 20 min). However, over the full 20-min immobilization period, the P_aCO_2 was lower (p <0.05, areas under the response curves, Figure 2B) than that of the control. Oxygen insufflation as sole support had an

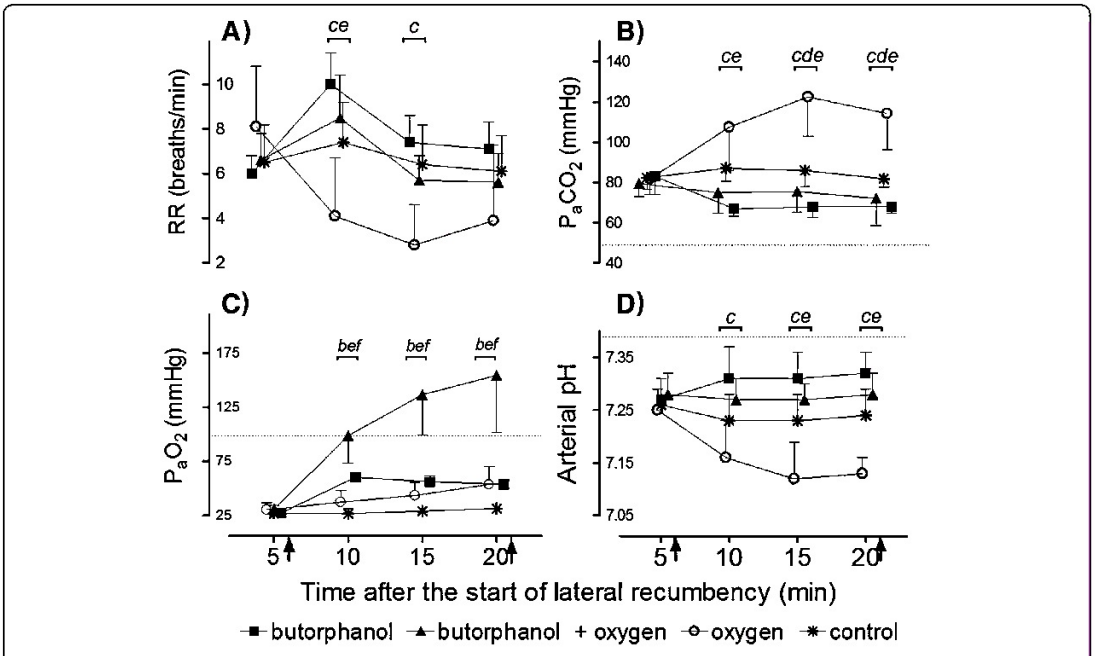


Figure 1 Values are respiratory rate (A), partial pressure of arterial carbon dioxide (P_aCO_2) (B), partial pressure of arterial oxygen (P_aO_2) (C), and arterial pH (D) (means, SD, n =8) of rhinoceros given intravenous butorphanol, intravenous butorphanol + oxygen insufflation, oxygen insufflation and intravenous sterile water (control) 6 minutes after the rhinoceros became laterally recumbent. Arrow at 6 minutes indicates time at which the intervention was given. Arrow at 21 minutes indicates the end of lateral recumbency. Dotted lines indicate average normal values in standing, unsedated white rhinoceros [12]. The brackets show when treatments differed from each other with letters indicating the treatment comparisons as follows: a = butorphanol vs. control; b = butorphanol vs. butorphanol + oxygen; c = butorphanol vs. oxygen; d = oxygen vs. control; e = oxygen vs. butorphanol + oxygen and f = butorphanol + oxygen vs. control.

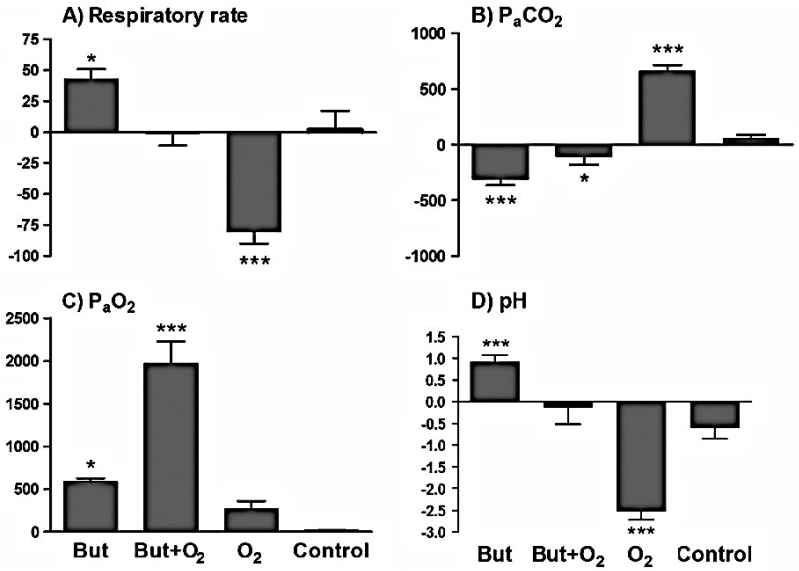


Figure 2 Areas under the response curves. Comparisons between the calculated values of the area under the response curves for respiratory rate **(A)**, partial pressure of arterial carbon dioxide (P_aCO_2) **(B)**, partial pressure of arterial oxygen (P_aO_2) **(C)** and arterial pH **(D)**. The asterix (*) indicates when the treatments butorphanol (But), butorphanol + oxygen (But + O_2) or oxygen only (O_2) differ significantly from the control, with the number of asterixes indicating the level of significance (*p <0.05, **p <0.01 and ***p <0.001).

immediate negative effect, increasing P_aCO_2 at 10 min to 106 ± 21 mmHg ($F_{(3,21)}$ =50.13, p <0.001). At 15 min, P_aCO_2 peaked at 123 ± 20 mmHg, about 50% higher than the control value (p <0.001) at that time point (Figure 1B).

Partial pressure of arterial oxygen (P_aO_2)

Chemical immobilization resulted in severe hypoxaemia (P_aO_2 = 27 ± 1 mmHg, normal =98.2 [±1.4] mmHg [12]) in the control trial at 5 min. This hypoxaemia at 5 min was similar across rhinoceros in all trials (butorphanol =28 ± 4 mmHg; butorphanol + oxygen =31 ± 6 mmHg; oxygen =30 ± 7 mmHg) (Figure 1C). Without any supportive treatment, oxygenation remained at extremely low levels (less than 35 mmHg) throughout the immobilization period. Butorphanol administration resulted in an immediate increase in P_aO_2 (60 ± 3 mmHg at 10 min; $F_{(3,21)}$ =151.9, p <0.001), with better oxygenation compared to the control over the whole immobilization period (p <0.05, areas under the response curves, Figure 2C), although normoxia was not attained. Butorphanol + oxygen resulted in a marked and sustained increase in P_aO_2 (99 ± 26 mmHg at 10 min, $F_{(3,21)}$ =33.36, p <0.001), which was greater than the control value at all time points (p <0.001) (Figure 1C). Indeed, butorphanol + oxygen increased P_aO_2 to levels above those normally seen in standing, unsedated white rhinoceros, and the increase was greater than butorphanol administration alone (butorphanol + oxygen =154 ± 53 mmHg; butorphanol =54 ± 4 mmHg at 20 min, p <0.001). Surprisingly, the overall improvement in P_aO_2 was greater than the combined effects of butorphanol alone and oxygen alone (Figure 2C). Administration of oxygen alone resulted in a delayed increase in P_aO_2 with an improvement seen only at 15 and 20 min (54 ± 17 mmHg, $F_{(3,21)}$ =7.82, p <0.05) compared to pre-intervention values. However, oxygen insufflation did not result in an overall improvement in P_aO_2 compared to the control (p >0.05, areas under the response curves, Figure 2C).

Arterial pH

Corresponding to the severe hypercapnia, animals that received the control intervention were acidaemic at 5 min (pH =7.26 ± 0.02, normal =7.39 ± 0.1 [12]) and their pH remained low throughout the immobilization period, with no difference between the pH at 5 min (7.26 ± 0.02) and 20 min (7.24 ± 0.05, $F_{(3,21)}$ =4.64, p >0.05). The initial acidaemia at 5 min was similar across all treatment groups (butorphanol =7.27 ± 0.04; butorphanol + oxygen =7.28 ± 0.04; oxygen =7.25 ± 0.04) (Figure 1D). When butorphanol was administered, arterial pH improved initially, with pH at 10 min (7.31 ± 0.06) higher (p <0.001) than the pre-intervention pH. However, this improvement was not sustained and the rhinoceros remained acidaemic throughout the immobilization period. Overall, butorphanol led to an improvement in

pH compared to the control (p <0.001, areas under the response curves, Figure 2D). Butorphanol + oxygen did not alter the pH at any time points following the intervention ($F_{(3,21)}$ =0.55, p =0.66) and over the full 20-min immobilization period, the pH was not different from the control (Figure 2D). Oxygen alone caused a drop in pH from 7.25 ± 0.04 at 5 min to 7.13 ± 0.03 at 20 min ($F_{(3,21)}$ =110.5, p <0.001), resulting in more severe acidaemia compared to the control and all other interventions (p <0.001, areas under the response curves, Figure 2D).

Arousal

The median arousal score for rhinoceros after immobilization with butorphanol was 2, (animal gets up easily after minor stimulation, and when standing can be blindfolded, safely handled and walked without stimulation); while that for the control, oxygen and butorphanol + oxygen was 3 (animal gets up after moderate stimulation and when standing can be blindfolded, safely handled and walked with stimulation). Thus, rhinoceros that received only butorphanol at 6 min were aroused more easily at 26 min compared to rhinoceros that received the other treatments. Following the full reversal with naltrexone, all rhinoceros had a good or average recovery and returned to their bomas without complication.

Arterial blood results from standing sedated rhinoceros

The last blood sample from the standing sedated rhinoceros while in the weighing crate, was taken, on average, 27 min (range 23–33 min) after the rhinoceros became recumbent. Following all the interventions, including the control, samples from the standing sedated rhinoceros indicated that the animals were still hypoxaemic (mean P_aO_2 values: control =69 ± 3 mmHg, butorphanol =58 ± 9 mmHg, butorphanol + oxygen =64 ± 8 mmHg, oxygen =87 ± 9 mmHg), hypercapnic (mean P_aCO_2 values: control =64 ± 4 mmHg, butorphanol =67 ± 3 mmHg, butorphanol + oxygen =67 ± 5 mmHg, oxygen =58 ± 4 mmHg) and acidaemic (mean pH values: control =7.33 ± 0.05, butorphanol =7.34 ± 0.03, butorphanol + oxygen =7.33 ± 0.02, oxygen =7.37 ± 0.05). However, the P_aO_2 values (87 ± 9 mmHg) from the animals that had received oxygen during immobilization, and then butorphanol one minute before walking, were higher than those in the butorphanol + oxygen (p <0.05) and butorphanol (p <0.01) groups.

Discussion

We have shown that butorphanol administration in recently immobilized white rhinoceros attenuates the respiratory derangements that typically occur after etorphine-induced immobilization. However, the animals were still critically hypoxaemic (P_aO_2 = 53 ± 4 mmHg, normal P_aO_2 = 98.2 ± 1.4 mmHg [12]) 20 minutes into the immobilization period. The drug-induced hypoxaemia in white rhinoceros, however, could be corrected completely by administering intravenous butorphanol shortly after recumbency and supplying oxygen throughout the immobilization period via nasotracheal intubation. As far as we are aware, this is the first report of a protocol to completely reverse the severe hypoxaemia associated with etorphine-induced immobilization in white rhinoceros. However, the animals remained hypercapnic and acidaemic, illustrating that the immobilization-induced respiratory derangements were not entirely corrected. Surprisingly, we found that oxygen administered directly into the trachea at a flow rate of 30 L/min as sole support, decreased respiratory rate and exacerbated hypercapnia. Moreover, partial pressure of arterial oxygen was not statistically different from that of rhinoceros in the control group.

Arterial blood gas analysis is the gold standard for assessing the adequacy of oxygenation and ventilation. Although P_aO_2 and P_aCO_2 could be accurately determined, due to equipment logistics (uncuffed oxygen administration tubing), measurement of fractional inspired oxygen (F_iO_2) during oxygen interventions would have been inaccurate due to mixing of air with delivered oxygen. Therefore, the alveolar-arterial pressure gradient ($P(A\text{-}a)O_2$) and other calculations could not be performed.

To our knowledge, this crossover trial is the first to critically evaluate, in a controlled environment, the respiratory physiology of the white rhinoceros during chemical immobilization. The effects of butorphanol [5,9] and oxygen insufflation [3] have been assessed previously during field immobilizations. However, as a result of uncontrolled variables (for example, dart placement, animal exertion) that occur in field trials, it is often difficult to establish definitive efficacy of a treatment, and comparing data from different field studies is thwart with error. Nevertheless, our study supports the common finding that etorphine immobilization in the white rhinoceros results in severe hypoxaemia together with hypercapnia and acidosis [1-3,5,7]. This hypoxaemia may be even more severe than previously reported, with rhinoceros sometimes having P_aO_2 values below 25 mmHg, less than a quarter of the normal value in an awake animal, five minutes into the immobilization period. Previously, the lowest reported P_aO_2 values were between 30 – 40 mmHg [2,3,5]. These negative effects of etorphine-induced immobilization on respiratory function are consistent with etorphine's action as a potent mu-opioid receptor agonist.

Butorphanol is a mixed opioid agonist–antagonist, which has either mu-antagonist or partial mu-agonist and kappa-agonist activity [13]. Use in white rhinoceros immobilization is based on anecdotal evidence that butorphanol improves respiration by reversing the etorphine-induced mu-receptor activation [8]. However, Miller and colleagues [9] recently suggested that the beneficial effect of butorphanol may be related to a change in

immobilization level rather than a direct antagonism of mu-receptors. Their conclusion was based on the persistence of hypoxaemia in rhinoceros given butorphanol during field immobilizations. In another study, immobilized white rhinoceros given nalorphine and diprenorphine, two other mixed opioid agonists-antagonists, also remained markedly hypoxaemic during immobilization [10]. Our finding that rhinoceros that received butorphanol 6 min after lateral recumbency were still hypoxaemic after 20 minutes of immobilization supports the idea that butorphanol does not reverse opioid-induced respiratory depression. However, in the rhinoceros in this study, ventilation did improve after butorphanol administration in comparison to the control, as indicated by lower P_aCO_2 and an improvement in P_aO_2. According to our 'level of immobilization score', butorphanol did not induce a lighter plane of immobilization compared to the control; however, we observed that some animals, although they remained completely immobile, were more responsive to gentle manipulation of the ear with this treatment. In future, additional criteria, such as response to a tactile stimulus, should be included in the 'level of immobilization' score. Butorphanol also appeared to reduce the etorphine-induced muscle tension and chest rigidity, an effect that may have accounted for improved minute ventilation.

In addition to butorphanol, another available treatment that has been advanced for improving oxygenation during rhinoceros immobilization is oxygen insufflation. In one study [3], the use of oxygen insufflation in field-immobilized white rhinoceros significantly increased arterial oxygen concentrations. Unexpectedly, our study contradicts these findings as we showed that oxygen as sole support did not improve P_aO_2 values compared to the control, and indeed had a detrimental effect on the respiratory status of immobilized white rhinoceros. This disparity in findings may be a result of intermittent use of a partial-opioid antagonist (nalorphine) and a respiratory stimulant (doxapram) during the field-based study [3], and other confounding variables associated with field trials.

Despite the variability between these studies, it is evident that hypoventilation, indicated by an increase in P_aCO_2, contributes to hypoxaemia in immobilized white rhinoceros. Another major contributing factor is likely to be ventilation-perfusion mismatch [1,5,10]. Previous studies have shown that $P(A\text{-}a)O_2$ values in immobilized, recumbent white rhinoceros are often high [5]. Moreover, atelectasis has been found to occur after prolonged immobilization and recumbency in a white rhinoceros [1], and intrapulmonary shunts are a well-described complication of anaesthesia and recumbency in another large perissodactyl, the horse [14,15]. Together with intrapulmonary shunts, lateral recumbency in large quadrapeds leads to ventilation of under-perfused alveoli resulting in increased alveolar dead space. Indeed, in black rhinoceros, alveolar dead space was found to be greater in lateral, compared to sternally recumbent black rhinoceros [16]. Recumbency therefore most likely played an important role in the respiratory derangements of the rhinoceros in our study, but was not the primary cause of respiratory depression. We believe the primary cause was opioid-induced respiratory depression as the animal's ventilatory response was inadequate to this severe hypoxaemia and hypercapnia.

The massive size of the rhinoceros undoubtedly contributes to atelectasis on the dependent side of the lung regions in laterally recumbent animals. However, absorption atelectasis, which occurs when less gas enters the alveolus than is removed by uptake by the blood, may be influenced by other factors such as gas composition [17]. The rate of absorption of gas from the alveoli into the blood increases with increasing F_iO_2 as oxygen is absorbed more quickly than nitrogen [17]. In humans with acute respiratory failure, mechanical ventilation with 100% oxygen causes an increase in intrapulmonary shunting primarily as a result of absorption atelectasis resulting from alveolar denitrogenation and pulmonary vasodilatation in unventilated lung areas [18-20]. Similarly, in horses under general anaesthesia where airway integrity is compromised by the weight of abdominal organs, increased intrapulmonary shunting occurs as a result of atelectasis when breathing high F_iO_2 (>0.8) compared to a lower F_iO_2 (0.21-0.3) [21,22]. It has also been demonstrated in horses receiving supplemental oxygen that lower minute ventilation will result in higher F_iO_2 [23]. Thus we propose that the rhinoceros receiving oxygen supplementation in our study had a high F_iO_2 together with narrowed or compressed airways as a result of pressure from abdominal organs and poor ventilation. In rhinoceros administered oxygen, gases in the alveoli were likely absorbed faster than they could be replaced and atelectasis ensued. If atelectasis is severe enough to cause shunt fractions in excess of 50%, oxygen supplementation will not be effective [10]. Therefore, we suggest that the rhinoceros receiving oxygen as the only intervention had significant intra-pulmonary shunt fractions, probably in the region of 50% or greater, as their P_aO_2 values were not significantly greater than the control.

When butorphanol was combined with oxygen insufflation, the partial pressure of arterial oxygen was substantially improved, and indeed entirely corrected with P_aO_2 reaching 154 ± 53 mmHg by the end of the recumbent period. This marked effect is difficult to explain given the negative effects of oxygen on its own, and the moderate improvements seen with butorphanol alone, but is most likely a result of the improved tidal volume and chest expansion induced by the butorphanol, which facilitated the high concentration of oxygen in the alveoli to dissolve into the blood without causing excessive atelectasis.

If atelectasis occurred in the rhinoceros that received oxygen only, it must have resolved quickly, as we found

that, in standing rhinoceros, after the oxygen had been discontinued, P_aO_2 (87 ± 9 mmHg) was relatively close to normal levels. At least in one study investigating humans with acute respiratory failure [20], the loss of functional lung volume that occurred as a result of ventilation with 100% oxygen for 20 min, was immediately reversible in 19 of 20 patients. In our study, butorphanol was administered at the end of the recumbent period in the rhinoceros that received oxygen as sole support, so it is also likely that the butorphanol, together with stimulation and getting the animal's into a standing position, led to improved ventilation and P_aO_2 values in these animals. On the other hand, rhinoceros that were given butorphanol at the beginning of the immobilization period, and were only stimulated to stand after 25 min, were still severely hypoxaemic (P_aO_2 = 58.4 ± 9.1 mmHg) in the standing position. This finding reveals that opioid-induced respiratory depression is a bigger complication than recumbency during white rhinoceros immobilization.

The partial pressure of arterial oxygen during oxygen administration may increase to a level at which hyperoxia occurs, thus affecting the ventilatory control system by reducing the output of peripheral and central chemoreceptors [24]. However, in our study, the rhinoceros that received inhalation oxygen as the sole support never became hyperoxic, but rather remained severely hypoxaemic. Normally, increasing levels of hypercapnia, as observed in these rhinoceros, should stimulate ventilation. However, full opioid agonists, such as etorphine, significantly reduce chemoreceptor sensitivity and therefore normal ventilatory control is inhibited during opioid-induced immobilization [13]. This inhibition of ventilatory control however, does not explain why the respiratory rate in rhinoceros receiving oxygen decreased from about 8 breaths per minute before oxygen administration to about 3 breaths per minute at 15 min when P_aCO_2 was at its highest (123 ± 12 mmHg) and P_aO_2 was only 44 ± 12 mmHg. Reasons for this further decrease in respiratory rate are not clear, but we hypothesize that it was directly related to the extreme hypercapnia resulting from a high shunt fraction, which caused a central depression of respiration [25].

This study has demonstrated that it is possible to fully correct the severe hypoxaemia that occurs in chemically-immobilized white rhinoceros. This is essential since inadequate tissue oxygenation may lead to an acute cessation of organ function resulting in morbidity, and even mortality [24]. However, although we managed to correct the hypoxaemia using butorphanol + oxygen, the intervention did not correct hypercapnia and acidaemia. Therefore, further investigations are needed to find treatments that fully support the respiratory physiology of immobilized white rhinoceros. Moreover, it is important to determine whether butorphanol combined with oxygen insufflation has the same positive effect on immobilization-induced hypoxaemia in field-immobilized white rhinoceros where increased exertion and other variables may alter individual responses.

Conclusions

We compared the efficacy of butorphanol and oxygen insufflation, alone or in combination, for reversing opioid-induced respiratory depression in the chemically immobilized white rhinoceros. Butorphanol alone, although partly effective, did not prevent hypoxaemia and hypercapnia. Oxygen insufflation also did not reverse hypoxaemia or hypercapnia. Indeed, oxygen alone severely compromised the animal's respiratory status, an effect we attribute to ventilation-perfusion mismatch and atelectasis.

Whilst the effects of butorphanol and oxygen administered alone were disappointing, oxygen insufflation combined with the intravenous administration of butorphanol at the beginning of the immobilization period completely reversed hypoxaemia. This supportive intervention therefore is likely to reduce, or even eliminate, the risk of hypoxic damage to vital organs and muscles, thereby improving the safety of white rhinoceros immobilization. Rhinoceros that received this treatment could be aroused with some stimulation, so that they could be guided into a loading crate, an important consideration when capturing large pachyderms. In the confined boma setting, where rhinoceros can be darted with ease and without excessive animal exertion, we recommend butorphanol and oxygen as the best available treatment to reverse hypoxaemia after etorphine administration. Whether the butorphanol and oxygen combination is equally effective in field-immobilized rhinoceros remains to be determined.

Abbreviations

E_TCO_2: End-tidal carbon dioxide concentration; P_aO_2: Partial pressure of oxygen in the arterial blood; P_aCO_2: Partial pressure of carbon dioxide in the arterial blood; S_aO_2: Percentage of haemoglobin saturated with oxygen as measured in an arterial blood sample; SD: Standard deviation; SNK: Student-Newman-Keuls.

Competing interests

The authors declare that they have no competing interests that could be perceived as prejudicing the impartiality of the research reported.

Authors' contributions

Study conception and design: AF, AH, GF, LM, MH, MM, PB. Data collection and experimental procedures: AF, AH, GF, LM, MH, MM, PB. Data analysis: AH, AF, LM. First draft: AH. Comments on draft and final approval: AF, AH, LM, MH, MM, PB. All authors read and approved the final manuscript.

Authors' information

This paper is dedicated to Dr Gregory Fleming in memory of his passion for wildlife research.

Acknowledgements

We thank the staff of the SANParks Veterinary Wildlife Services for care and management of the boma-kept rhinoceros and for dedicated support and assistance with experimental procedures. In particular we thank Johan Malan

for coordinating the husbandry of the rhinoceros, Marius Kruger for managing the capture team and Jennifer Hofmeyr, Nomkhosi Mathebula and Guy Hausler for field and laboratory assistance. We also thank the South African National Research Foundation, the International Rhino Foundation, Disney's Animal Programs and Environmental Initiatives, SANParks, the South African Veterinary Association's Wildlife Group and the University of the Witwatersrand for financial support.

Author details
[1]Brain Function Research Group, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. [2]Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa. [3]Veterinary Wildlife Services, South African National Parks, Kruger National Park, Skukuza, South Africa. [4]Division of Molecular Biology and Human Genetics, Faculty of Health Sciences, Stellenbosch University, Stellenbosch, South Africa. [5]Disney's Animal Programs and Environmental Initiatives, Lake Buena Vista, Florida, USA. [6]Centre for Veterinary Wildlife Studies, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa.

Received: 30 May 2014 Accepted: 9 October 2014
Published online: 15 October 2014

References

1. Heard DJ, Olsen JH, Stover J: **Cardiopulmonary changes associated with chemical immobilization and recumbency in a white rhinoceros (Ceratotherium simum).** *J Zoo Wildl Med* 1992, **23**:197–200.
2. Hatting J, Knox CM: **Arterial blood pressure and blood gas composition of white rhinoceroses under etorphine anaesthesia.** *S Afr J Wildl Res* 1994, **24**:12–14.
3. Bush M, Raath JP, Grobler D, Klein L: **Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen.** *J S Afr Vet Assoc* 2004, **72**:79–84.
4. Portas TJ: **A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species.** *Aust Vet J* 2004, **2004**(82):542–549.
5. Wenger S, Boardman W, Buss P, Govender D, Foggin C: **The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field-anaesthetized white rhinoceroses (*Ceratotherium simum*).** *J Zoo Wildl Med* 2007, **38**:380–387.
6. Burroughs R, Meltzer D, Morkel P: **Applied pharmacology.** In *Chemical and Physical Restraint of Wild Animals*. 2nd edition. Edited by Kock MD, Meltzer D, Burroughs R. South Africa: IWVS (Africa); 2012:53–62.
7. Kock MD, Morkel P, Atkinson M, Foggin C: **Chemical immobilization of free-ranging white rhinoceros (*Ceratotherium simum simum*) in Hwange and Matobo National Parks, Zimbabwe, using combinations of etorphine (M99), fentanyl, xylazine and detomidine.** *J Zoo Wildl Med* 1995, **26**:207–219.
8. Burroughs R, Hofmeyr M, Morkel P, Kock MD, Kock R, Meltzer D: **Chemical immobilization – individual species requirements.** In *Chemical and Physical Restraint of Wild Animals*. 2nd edition. Edited by Kock MD, Meltzer D, Burroughs R. South Africa: IWVS (Africa); 2012:223–234.
9. Miller M, Buss P, Joubert J, Mathebula N, Kruger M, Martin L, Hofmeyr M, Olea-Popelka F: **Use of butorphanol during immobilization of free-ranging white rhinoceros (*Ceratotherium simum*).** *J Zoo Wildl Med* 2013, **44**:55–61.
10. Fahlman A: *Advances in Wildlife Immobilization and Anaesthesia: Clinical and Physiological Evaluation in Selected Species. PhD Thesis*. Uppsala: Swedish University of Agricultural Sciences; 2008.
11. Mathebula N, Miller M, Buss P, Joubert J, Martin L, Kruger M, Hofmeyr M, Olea-Popelka F: **Biochemical values in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa.** *J Zoo Wildlife Med* 2012, **43**:530–538.
12. Citino SB, Bush M: **Reference cardiopulmonary physiologic parameters for standing, unrestrained white rhinoceroses (*Ceratotherium simum*).** *J Zoo Wildl Med* 2007, **38**:375–379.
13. Kukanich B, Papich MG: **Opioid analgesic drugs.** In *Veterinary Pharmacology and Therapeutics*. 9th edition. Edited by Riviere JE, Papich MG. Iowa: Blackwell Publishing; 2009.
14. Nyman G, Funkquist B, Kvart C, Frostell C, Tokics L, Strandberg Å, Lundquist H, Lundh B, Brismar B, Hedenstierna G: **Atelectasis causes gas exchange impairment in the anaesthetized horse.** *Equine Vet J* 1990, **22**:317–324.
15. Thurmon JC: **General clinical considerations for anesthesia of the horse.** *Vet Clin N Am - Equine* 1990, **6**(3):485–494.
16. Morkel P, Radcliffe RW, Jago M, du Preez P, Flaminio MJBF, Nydam DV, Taft A, Lain D, Miller MM, Gleed RD: **Acid–base balance and ventilation during sternal and lateral recumbency in field immobilized black rhinoceros (Diceros bicornis) receiving oxygen insufflation: a preliminary report.** *J Wildl Dis* 2010, **46**:236–245.
17. Magnusson L, Spahn DR: **New concepts of atelectasis during general anaesthesia.** *Brit J Anaesth* 2003, **91**:61–72.
18. Santos C, Ferrer M, Roca J, Torres A, Hernandez C, Rodriguez-Roisin R: **Pulmonary gas exchange response to oxygen breathing in acute lung injury.** *Am J Resp Crit Care* 2000, **161**:26–31.
19. Aboab J, Jonson B, Kouatchet A, Taille S, Niklason L, Brochard L: **Effect of inspired oxygen fraction on alveolar derecruitment in acute respiratory distress syndrome.** *Intens Care Med* 2006, **32**:1979–1986.
20. Suter P, Fairley H, Schlobohm R: **Shunt, lung volume and perfusion during periods of ventilation with oxygen.** *Anesthesiolog* 1975, **43**:617–627.
21. Marntell S, Nyman G, Hedenstierna G: **High inspired oxygen concentrations increase intrapulmonary shunt in anaesthetized horses.** *Vet Anaesth Analg* 2005, **32**:338–347.
22. Staffieri F, Bauguier SH, Moate PJ, Driessen B: **Pulmonary gas exchange in anesthetised horses mechanically ventilated with oxygen or helium/oxygen mixture.** *Equine Vet J* 2009, **41**(8):747–752.
23. Crumley MN, Hodgson DS, Kreider SE: **Effects of tidal volume, ventilator frequency, and oxygen insufflation flow on the fraction of inspired oxygen in cadaveric horse heads attached to a lung model.** *Am J Vet Res* 2012, **73**:134–139.
24. McDonell W: **Respiratory system.** In *Lumb and Jones' Veterinary Anesthesia*. 3rd edition. Edited by Thurmon JC, Tranquilli WJ, Benson GJ. Baltimore: Williams and Wilkins; 1996:115–147.
25. Merriman JE: **The role of carbon dioxide in anaesthesia.** *Can Anaesth Soc J* 1955, **2**(2):273–280.

doi:10.1186/s12917-014-0253-0
Cite this article as: Haw *et al.*: Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*). *BMC Veterinary Research* 2014 **10**:253.

Submit your next manuscript to BioMed Central and take full advantage of:

- **Convenient online submission**
- **Thorough peer review**
- **No space constraints or color figure charges**
- **Immediate publication on acceptance**
- **Inclusion in PubMed, CAS, Scopus and Google Scholar**
- **Research which is freely available for redistribution**

Submit your manuscript at
www.biomedcentral.com/submit

BioMed Central

CHAPTER 4

Improved cardiorespiratory function in immobilised wild white rhinoceros (*Ceratotherium simum*)

Haw A, Hofmeyr M, Fuller A, Buss P, Miller M, Fleming G and Meyer L

***Journal of the South African Veterinary Association* 86:E1-E10, 2015**

Butorphanol with oxygen insufflation improves cardiorespiratory function in field-immobilised white rhinoceros (*Ceratotherium simum*)

Authors:
Anna Haw[1]
Markus Hofmeyr[2]
Andrea Fuller[1]
Peter Buss[2]
Michele Miller[3]
Gregory Fleming[4]†
Leith Meyer[5]

Affiliations:
[1]School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, South Africa

[2]South African National Parks, Skukuza, South Africa

[3]Division of Molecular Biology and Human Genetics, DST/NRF Centre of Excellence for Biomedical Tuberculosis, Stellenbosch University, South Africa

[4]Disney's Animal Programs and Environmental Initiatives, Lake Buena Vista, United States

[5]Department of Paraclinical Sciences, University of Pretoria, Onderstepoort, South Africa

Correspondence to:
Anna Haw

Email:
anna.haw@wits.ac.za

Postal address:
7 York Road, Parktown 2193, Johannesburg, South Africa

Dates:
Received: 03 Feb. 2015
Accepted: 03 June 2015
Published: 12 Aug. 2015

Read online:
Scan this QR code with your smart phone or mobile device to read online.

Opioid-induced immobilisation results in severe respiratory compromise in the white rhinoceros (*Ceratotherium simum*). The effectiveness of oxygen insufflation combined with butorphanol in alleviating respiratory depression in free-ranging chemically immobilised white rhinoceroses was investigated. In this prospective intervention study 14 free-ranging white rhinoceroses were immobilised with a combination of etorphine, azaperone and hyaluronidase. Six minutes (min) after the animals became recumbent, intravenous butorphanol was administered and oxygen insufflation was initiated. Previous boma trial results were used for comparison, using repeated measures two-way analysis of variance. The initial immobilisation-induced hypoxaemia in free-ranging rhinoceroses (arterial partial pressure of oxygen [P_aO_2] 35.4 mmHg ± 6.6 mmHg) was similar to that observed in boma-confined rhinoceroses (P_aO_2 31 mmHg ± 6 mmHg, n = 8). Although the initial hypercapnia (P_aCO_2 63.0 mmHg ± 7.5 mmHg) was not as severe as that in animals in the boma trial (79 mmHg ± 7 mmHg), the field-immobilised rhinoceroses were more acidaemic (pH 7.10 ± 0.14) at the beginning of the immobilisation compared with boma-immobilised rhinoceroses (pH 7.28 ± 0.04). Compared with pre-intervention values, butorphanol with oxygen insufflation improved the P_aO_2 (81.2 mmHg ± 23.7 mmHg, $p < 0.001$, 5 min vs 20 min), arterial partial pressure of carbon dioxide (55.3 mmHg ± 5.2 mmHg, $p < 0.01$, 5 min vs 20 min), pH (7.17 ± 0.11, $p < 0.001$, 5 min vs 20 min), heart rate (78 breaths/min ± 20 breaths/min, $p < 0.001$, 5 min vs 20 min) and mean arterial blood pressure (105 mmHg ± 14 mmHg, $p < 0.01$, 5 min vs 20 min). Oxygen insufflation combined with a single intravenous dose of butorphanol improved oxygenation and reduced hypercapnia and acidaemia in immobilised free-ranging white rhinoceroses.

Introduction

A key component of the conservation of white rhinoceroses (*Ceratotherium simum*) relies on the ability to safely immobilise these large pachyderms for translocation, dehorning, identity marking and veterinary treatments. Potent mu-agonist opioids, such as etorphine hydrochloride, are the only category of drugs capable of inducing rapid and reversible immobilisation of free-ranging white rhinoceroses. However, a drawback of use of these potent opioids is respiratory depression characterised by hypoventilation, hypoxaemia, hypercapnia and acidaemia (Burroughs, Meltzer & Morkel 2012; Bush *et al.* 2004; Hattingh & Knox 1994; Heard, Olsen & Stover 1992; Portas 2004; Wenger *et al.* 2007).

The massive size of the white rhinoceros, and in particular its large digestive tract together with muscle rigidity are thought to be significant factors in reducing the ability of the animal to breathe adequately during recumbency (Cornick-Seahorn *et al.* 1995). As a result, potentially life-threatening hypoxaemia is one of the most significant side-effects in etorphine-immobilised free-ranging white rhinoceroses (Fahlman, Foggin & Nyman 2004; Heard *et al.* 1992; Kock *et al.* 1995; Raath 1999).

In a series of controlled experiments in captive white rhinoceroses housed in bomas (small enclosures to hold wild animals temporarily) it was demonstrated that intravenous butorphanol administered at the beginning of the immobilisation period, together with oxygen insufflation supplied throughout immobilisation, provided the best cardiorespiratory-supportive treatment for immobilised, laterally recumbent rhinoceroses (Haw *et al.* 2014). However, outside the

How to cite this article: Haw, A., Hofmeyr, M., Fuller, A., Buss, P., Miller, M., Fleming, G. *et al.*, 2015, 'Butorphanol with oxygen insufflation improves cardiorespiratory function in field-immobilised white rhinoceros (*Ceratotherium simum*)', *Journal of the South African Veterinary Association* 86(1), Art. #1276, 10 pages. http://dx.doi.org/10.4102/jsava.v86i1.1276

Note: †13 March 1966 – 09 March 2013.

Copyright: © 2015. The Authors. Licensee: AOSIS OpenJournals. This work is licensed under the Creative Commons Attribution License.

experimental setting most rhinoceroses are wild caught in the field. The physiology of field-immobilised rhinoceroses may differ substantially from that of boma-kept animals, particularly if exercise and additional stress are imposed by a vehicle or helicopter chase to which an animal is not accustomed. It is therefore not known whether a cardiorespiratory treatment found to be effective in immobilised, boma-kept rhinoceroses would be as effective in free-ranging rhinoceros.

The aim of the present study was to establish whether butorphanol combined with oxygen insufflation would afford the same positive results in field-immobilised rhinoceroses as it did in boma-kept white rhinoceroses.

Research method and design

Study area and sample population

The study animals were 14 sub-adult (3–7 year-old) male white rhinoceroses, which were immobilised in Kruger National Park (-24.98984 S, 31.59263 E, altitude 317 m) during February 2013. All immobilisations took place between 06:00 and 12:30 and ambient temperature was between 22 °C and 32 °C. Barometric pressure, measured by the on-board barometer of the blood gas analyser, ranged from 717.1 mmHg to 748.3 mmHg. All animals appeared healthy based on body condition, which was confirmed on retrospective haematology and serum biochemical analyses.

Design

Fourteen rhinoceroses were immobilised with a combination of 2.0 mg – 3.5 mg etorphine hydrochloride (M99®, Novartis, Kempton Park, South Africa, 9.8 mg/mL), 30.0 mg – 52.5 mg azaperone (Stressnil®, Janssen Pharmaceuticals Ltd, Halfway House, South Africa, 40 mg/mL), and 2500 IU hyaluronidase (lyophilised hyalase, Kyron Laboratories, Benrose, South Africa). Each rhinoceros was located and darted from a helicopter, and drug doses were determined by an experienced veterinarian (M.H.) based on estimated body mass.

Immobilising drugs were administered using a 3 mL plastic dart with a 60 mm needle, fired by a carbon dioxide-powered dart-gun (Dan-Inject, International S.A., Skukuza, South Africa). Once the animal was safe to handle a ground crew approached, placed a blindfold on the rhinoceros, and positioned the animal in lateral recumbency (time 0), elevating the head with padding to ensure that the lower nostril remained patent for unobstructed ventilation. One observer in the helicopter estimated the distance that the rhinoceros ran before and after darting by counting 100 m segments on the ground with the aid of the helicopter's global positioning system. Signs of induction from darting to recumbency, including slowing and ataxia, were observed from the helicopter and times recorded.

If an animal was not recumbent within 15 min of darting it was excluded from the study and the effects of the immobilising agent immediately reversed. The speed at which the rhinoceros ran after dart placement was calculated as the estimated distance (metres) travelled divided by the time (seconds) it took for the animal to come to a complete halt.

Each rhinoceros received intravenous butorphanol (15 mg/mg etorphine) 6 min after it was positioned in lateral recumbency, combined with tracheal oxygen insufflation (30 L/min, see details on administration below); oxygen insufflation started at 6 min and continued throughout the recumbent period. Clinical data and samples were collected at 5 min after animals became laterally recumbent (1 minute before the intervention) and every 5 min thereafter for a 25-minute immobilisation period.

Clinical monitoring and data collection

Heart rate was measured directly by auscultation and counting the number of beats over one minute. Body temperature was measured with a thermocouple probe inserted 10 cm into the rectum and connected to a thermometer (BAT-12, Physitemp Instruments Clifton, New Jersey, United States of America [USA]). Respiratory rate was measured by observing and counting thoracic and abdominal excursions and feeling for expired air at the nares, over one minute.

The level of immobilisation was assessed by a veterinarian (M.H.) observing whole-body and ear movements, which were scored at 5, 10, 15, 20 and 25 min. The immobilisation score ranged from 1 (no immobilising or sedative effect) to 6 (excessive immobilisation depth with respiration < 3 breaths/min). Level 3 indicated a safe standing sedation, whilst levels 4 and 5 indicated recumbent immobilisation with or without ear movement, respectively.

The medial auricular artery was catheterised using a catheter (22G × 1", Nipro Safelet Cath, Nipro Corporation) secured in place with superglue (SuperGlue, Loctite®). A three-way stopcock valve with 10 cm compliant tube extension (Mediflexo, Eastern Medikit Ltd) was attached to the catheter to allow continuous direct blood pressure measurements via a pressure transducer (Deltran II, Utah Medical Products Inc., USA) attached to a custom-made portable processor (IntraTorr, Edenvale, South Africa) and easy access for arterial samples. A 0.5 mL sample was collected anaerobically into 1 mL pre-heparinised syringes at 5, 10, 15, 20 and 25 min after the rhinoceros became laterally recumbent.

Arterial pH, partial pressure of carbon dioxide (P_aCO_2), partial pressure of oxygen (P_aO_2) and lactate were measured, whilst haemoglobin oxygen saturation (S_aO_2) and bicarbonate (HCO_3) were calculated immediately, using a portable precalibrated blood gas analyser with self-calibrated test cards (EPOC® Portable analyser system and EPOC® BGEM test cards, Kyron Laboratories, Johannesburg, South Africa). The blood gas variables were reported at 37 °C. The catheter was maintained with a heparinised (heparin 5000 IU/mL; Fresenius, Port Elizabeth, South Africa) saline flush and 1 mL – 2 mL of blood was discarded before collection of each sample.

Oxygen (100% medical oxygen, African Oxygen Limited [Afrox], Johannesburg, South Africa) was delivered at 30 L/min using an equine stomach tube (9.5 mm outer diameter × 213 cm, Kyron Laboratories) inserted nasotracheally as described previously (Bush *et al.* 2004). The tube was inserted to a predetermined length, which ensured delivery of oxygen into the distal end of the trachea; air movement was felt to verify that the tube was in the trachea and not the oesophagus. Custom-designed tubing (Hospital Servicing Consultants cc, Lonehill, South Africa) was used to connect the stomach tube to two 15 L/min oxygen flow metres operating in parallel.

Silver duct tape was applied to the rhinoceros' horn and back leg to mark the animal temporarily and ensure that it was not re-immobilised during the study.

Twenty-six min into the recumbent period the rhinoceros was stimulated to stand and guided into a crate for weighing. Quality of arousal was assessed by M.H. according to the amount of stimulation needed to get the animal into a standing position and the ease with which the animals could be guided into a crate. The score was based on an incremental scale from 1 (animal gets up quickly, too awake and cannot be safely handled) to 5 (animal struggles to get up even after excessive stimulation, is unable to walk and becomes recumbent). A score of 3 (animal gets up after moderate stimulation and when standing can be safely handled and walked with stimulation) was considered ideal.

About 40 min after initial recumbency the effects of etorphine were reversed using the full-opioid antagonist naltrexone (Naltrexone, 50 mg/mL, Kyron Laboratories, Johannesburg), administered intravenously into an auricular vein at 20 mg/mg etorphine. The rhinoceros was then released from the weighing crate.

Comparison to boma-held rhinoceroses

Previously reported findings from a study on eight individually housed sub-adult male white rhinoceroses that each received four different treatment interventions at two-week intervals were used for comparison (Haw *et al.* 2014). Each rhinoceros was immobilised with the same drug combinations and dose rates as used in this field study. However, the boma-kept rhinoceroses were unobtrusively darted from a platform above each boma, rather than from a helicopter. Similar to this field study, treatment interventions in the boma trial were administered 6 min after the rhinoceros became laterally recumbent and comprised: (1) intravenous butorphanol (15 mg/mg etorphine), (2) tracheal oxygen insufflation at 30 L/min, which started at 6 min and continued throughout the immobilisation period, (3) intravenous butorphanol (15 mg/mg etorphine) combined with tracheal oxygen insufflation (butorphanol + oxygen) and (4) intravenous sterile water (control).

Arterial samples for blood gas analyses and non-invasive cardiorespiratory measurements (respiratory rate and heart rate) were taken before the intervention, at 5 min, and every 5 min thereafter for 20 min during the immobilisation period, using the same methods described above. The blood gas analyser used in the boma study (Roche OPTI CCA Analyzer and OPTI cassette B, Kat Medical, Johannesburg) was different from that used in this field study, which recorded additional variables such as plasma lactate concentration. In order to test agreement between the two analysers, simultaneous measurements from 35 samples obtained from immobilised rhinoceroses were compared. P_aO_2, P_aCO_2, pH and HCO_3 values from each analyser were significantly and strongly correlated ($p < 0.0001$; $r^2 = 0.97, 0.74, 0.97$ and 0.94 for P_aO_2, P_aCO_2, pH and HCO_3 respectively). The mean bias and limits of agreements between each analyser for each variable were small relative to the statistical differences found in Figures 1 and 2; thus values obtained from each analyser were used without correction.

As butorphanol + oxygen yielded the most favourable results in the boma study, this same protocol was retested in the field study. For logistical and ethical reasons (the boma trials showed critically abnormal physiological parameters in control animals) a control trial was not conducted in the field. Therefore results recorded from this field trial were compared with the boma treatment (butorphanol + oxygen) group and the boma control (sterile water) group.

Data analysis

Statistical analyses were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA). All results are expressed as mean ± standard deviation (s.d.) and $p < 0.05$ was considered significant. For P_aO_2, P_aCO_2, pH, respiratory rate and heart rate an unpaired repeated measures two-way analysis of variance (ANOVA) followed by Bonferroni post-tests was used to test for differences between responses to butorphanol + oxygen in field-immobilised rhinoceros, butorphanol + oxygen in boma-immobilised rhinoceros, and sterile water (control) in boma-immobilised rhinoceros at 5, 10, 15 and 20 min and between time points for each intervention. Although field-immobilised rhinoceroses were recumbent for 25 min, data are represented for the first 20 min of lateral recumbency, to allow for direct comparison to the boma study in which rhinoceroses were recumbent for only 20 min.

Pearson's correlation was performed to test for a relationship between plasma lactate at 5 min and the average estimated distance travelled by and average calculated running speed of each rhinoceros after the dart was fired. A repeated measures one-way ANOVA followed by Tukey's multiple comparison was used to test for differences in lactate concentrations at 5, 10, 15 and 20 min in the field-immobilised rhinoceroses.

Results

Rectal temperatures for field-immobilised rhinoceros ranged from 37.2 °C to 39.2 °C. The average body mass of the 14 field-captured rhinoceroses was 1272 kg ± 227 kg and each rhinoceros was estimated to be in the correct body mass bracket according to the body mass to dose table

(Haw *et al.* 2014). No mortality occurred in the rhinoceroses that were studied.

Induction period

Free-ranging rhinoceroses ran an estimated average distance of 1400 m ± 800 m before darting. The helicopter was used to herd rhinoceroses before dart placement, to ensure that once they were darted a ground crew could easily access the immobilised animals. Once darted the rhinoceros ran a further 1300 m ± 300 m before halting. The total combined distance ranged from 1000 m to 4400 m. The estimated speed the rhinoceros ran from darting to halting ranged from 2.5 m/s to 8.3 m/s (average 4.9 m/s ± 1.9 m/s). The administration of etorphine, azaperone and hyaluronidase led to rapid immobilisation of free-ranging rhinoceros, with recumbency attained in 7 min ± 2 min after darting. The time to recumbency was similar in the boma-immobilised rhinoceroses in both the control (5 min ± 1 min) and the butorphanol + oxygen trials (6 min ± 1 min). Throughout the recumbent period all field-immobilised rhinoceroses had an immobilisation level of 4 (recumbent with occasional ear movements). Both the treatment and control boma-immobilised rhinoceroses also had a median immobilisation score of 4.

Respiratory rate

Immobilisation caused hypopnoea (13 breaths/min ± 2 breaths/min, normal range 16–23 breaths/min [Citino & Bush 2007], Figure 1a) in field-immobilised rhinoceroses. After the administration of butorphanol and oxygen (at 6 min) there was an initial improvement in respiratory rate (15 breaths/min ± 2 breaths/min, $p < 0.01$, 5 min vs. 10 min, Figure 1a), but this improvement was short-lived with the respiratory rate decreasing again to 13 breaths/min ± 2 breaths/min at 15 min. A similar pattern in respiratory rate was observed in boma-immobilised rhinoceroses given the same supportive treatment (Figure 1a). However, the hypopnoea in the boma-immobilised rhinoceroses (treatment group; 7 breaths/min ± 2 breaths/min at 5 min, $p < 0.001$) was more severe than in the field. The respiratory rate of boma-immobilised rhinoceroses given butorphanol + oxygen was not different from that of boma-immobilised rhinoceroses that did not receive any supportive treatment (control). Overall, immobilised rhinoceroses in the field (given butorphanol + oxygen) had respiratory rates about twice those of boma-immobilised rhinoceros in both the treatment (butorphanol + oxygen) and control groups, throughout the immobilisation period [$F_{(2,27)} = 62.41, p < 0.001$].

Partial pressure of arterial carbon dioxide (P_aCO_2)

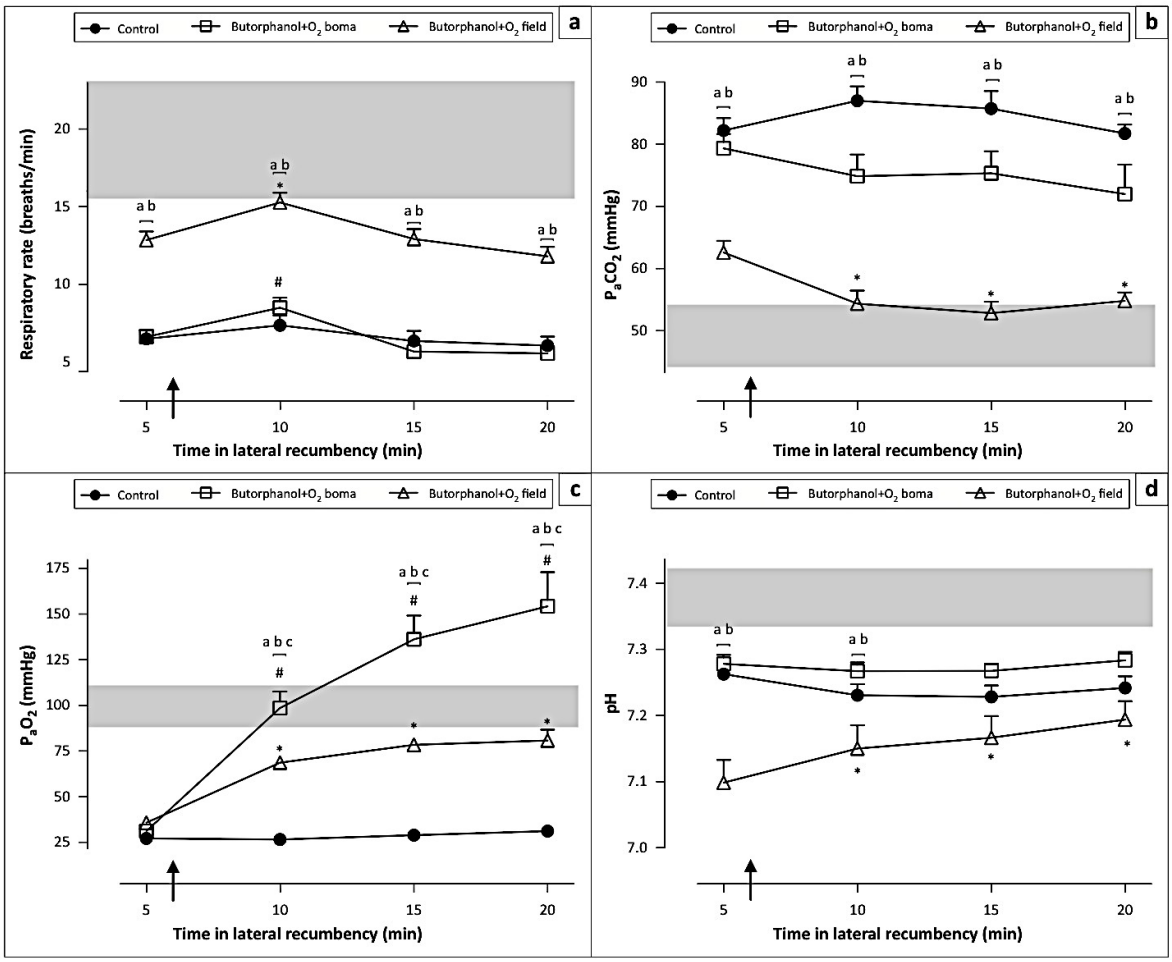
Field-immobilised rhinoceroses were hypercapnic at 5 min (63.0 mmHg ± 7.5 mmHg, normal range 44.4 mmHg – 53.7 mmHg [Citino & Bush 2007], Figure 1b). Administration of butorphanol with oxygen insufflation led to an immediate decrease in P_aCO_2 (54.7 mmHg ± 8.5 mmHg at 10 min, $p < 0.01$ vs. 5 min, Figure 1b). This lower arterial carbon dioxide concentration was sustained throughout the immobilisation period (P_aCO_2 at 20 min 55.3 mmHg ± 5.2 mmHg, $p < 0.01$, 5 min vs. 20 min). P_aCO_2 values in the field-immobilised rhinoceroses were also lower [$F_{(2,28)} = 60$, $p < 0.001$] than those of the boma control and treatment (butorphanol + oxygen) trials at all time points, including the period before the treatment intervention was administered. In boma-immobilised rhinoceroses butorphanol + oxygen did not lower the P_aCO_2 concentration compared with pre-intervention values [$F_{(3,21)} = 1.3$, $p = 0.3$], and there was no difference in the arterial carbon dioxide concentrations between boma-kept animals in the control and treatment (butorphanol + oxygen) groups.

Partial pressure of arterial oxygen (P_aO_2)

Chemical immobilisation led to severe hypoxaemia at 5 min (P_aO_2 35.4 mmHg ± 6.6 mmHg, normal range 90.2 mmHg – 108.6 mmHg [Citino & Bush 2007], Figure 1c) in field-immobilised white rhinoceroses. A similar degree of hypoxaemia was observed in boma-immobilised rhinoceroses (P_aO_2 31 mmHg ± 6 mmHg, $n = 8$, Figure 1c). Butorphanol combined with oxygen insufflation had an immediate positive effect on oxygenation in field-immobilised rhinoceroses (P_aO_2 at 10 min 68.9 mmHg ± 7.2 mmHg, $p < 0.01$, 5 vs. 10 min, Figure 1c) and improved arterial oxygenation compared with control boma-immobilised rhinoceroses at each time point following the intervention ($p < 0.05$ at 10 min; $p < 0.01$ at 15 min and 20 min). Similarly, butorphanol + oxygen had a profound positive effect in boma-immobilised rhinoceroses (P_aO_2 at 10 min 99 mmHg ± 26 mmHg, $n = 8$, Figure 1c), completely correcting hypoxaemia, with P_aO_2 values reaching 154 mmHg ± 53 mmHg 20 min into the immobilisation period. However, in the field-immobilised rhinoceroses given the same treatment (butorphanol + oxygen), oxygenation was not completely corrected (81.2 mmHg ± 23.7 mmHg at 20 min), with only three out of 14 rhinoceros achieving arterial oxygen concentrations greater than 90 mmHg at 20 min.

Arterial pH

Five min into the recumbent period all field-immobilised rhinoceroses were acidaemic (pH 7.10 ± 0.14, normal range 7.35–7.43 [Citino & Bush 2007], Figure 1d). Similar initial acidaemia was observed in boma-immobilised rhinoceroses (pH 7.28 ± 0.04 at 5 min), but was not as severe as in the field-immobilised rhinoceroses ($p < 0.05$, field vs. boma rhinoceroses at 5 min). In the field pH improved after administration of butorphanol + oxygen, with all pH values higher [$F_{(3,39)} = 34.57$, $p < 0.01$, Figure 1d] than pre-intervention values. However, at 20 min the animals were still acidaemic (pH 7.17 ± 0.11). The pH in boma-immobilised rhinoceroses given the same supportive intervention as those in the field did not improve [$F_{(3,21)} = 0.55$, $p = 0.66$], resulting in no difference in arterial pH between the field- and boma-immobilised rhinoceroses at 20 min ($p > 0.05$). Similarly, field-immobilised rhinoceroses were initially more acidaemic than the control group in the boma ($p < 0.01$), but by the end of the immobilisation period (20 min) there was no difference in



Note: Mean and s.d. of respiratory rate (a), P_aCO_2 (b), P_aO_2 (c) and arterial pH (d), of field-immobilised rhinoceroses given intravenous butorphanol + oxygen insufflation (butorphanol + O_2 field, n = 14), boma-immobilised rhinoceroses given intravenous butorphanol + oxygen insufflation (butorphanol + O_2 boma, n = 8) and boma-immobilised rhinoceroses given intravenous sterile water (control, n = 8). The black arrow indicates the time at which butorphanol or sterile water was administered and oxygen insufflation initiated. The asterisk (*) indicates values in the field trial that differed from pre-intervention values (5 min), and # indicates values in the boma treatment (butorphanol + O_2) trial that differed from pre-intervention values (5 min). The brackets indicate the time points at which the butorphanol + oxygen field trial differed from the control (a), the field trial differed from the butorphanol + oxygen boma trial (b), or the butorphanol + oxygen boma trial differed from the control (c). The shaded area represents the range of normal values in standing, unrestrained white rhinoceroses (Citino & Bush 2007).

FIGURE 1: (a) Respiratory rate, (b) P_aCO_2, (c) P_aO_2 and (d) pH.

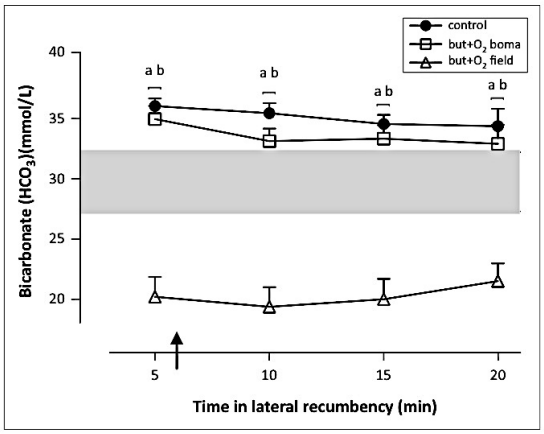
pH between the field-immobilised treatment group and the control group in the boma ($p > 0.05$).

Bicarbonate and lactate

Throughout the immobilisation period field-immobilised rhinoceroses had significantly lower bicarbonate concentrations than both the treatment and control boma-immobilised rhinoceroses [$F_{(2,27)} = 36.99, p < 0.0001$] (Figure 2). At the beginning of the immobilisation period 12 of 14 (86%) of the rhinoceroses in the field had high (above 5 mmol/L) lactate concentrations. The lactate concentrations in the rhinoceroses decreased during the immobilisation period [$F_{(2,25)} = 5.02$, $p = 0.02$], with values at 15 min (10.3 mmol/L ± 5.0 mmol/L) and 20 min (9.6 mmol/L ± 5.0 mmol/L) lower than those at 10 min (11.4 mmol/L ± 5.2 mmol/L) ($p < 0.05$, Figure 3a). Plasma lactate concentration was not correlated to the estimated distance run by the rhinoceros after dart placement ($p = 0.76$, $r^2 = 0.008$, Figure 3b). However, the average speed at which the rhinoceros ran after dart placement was positively correlated to plasma lactate concentration at 5 min ($p = 0.01$, $r^2 = 0.42$, Figure 3c).

Heart rate

Both field- and boma-immobilised rhinoceroses were tachycardic 5 min after initial recumbency (field-immobilised rhinoceroses 136 beats/min ± 34 beats/min, boma-immobilised rhinoceroses 141 beats/min ± 6 beats/min; normal range 32 beats/min – 42 beats/min, Figure 4a). The tachycardia was attenuated following administration of butorphanol + oxygen in field-immobilised rhinoceroses (heart rate 99 beats/min ± 30 beats/min at 10 min, $p < 0.01$, 5 min vs. 10 min). This decrease in heart rate was also observed in boma-immobilised rhinoceroses given the same treatment intervention (heart rate 92 beats/min ± 20 beats/min at 10 min,



Note: Mean and s.d. of bicarbonate concentration of field-immobilised rhinoceroses given intravenous butorphanol + oxygen insufflation (but + O_2 field, n = 14), boma-immobilised rhinoceroses given intravenous butorphanol + oxygen insufflation (but + O_2 boma, n = 8) and boma-immobilised rhinoceroses given intravenous sterile water (control, n = 8). The black arrow indicates the time at which butorphanol or sterile water was administered and oxygen insufflation initiated. The brackets indicate the time points at which the butorphanol + oxygen field trial differed from the control (a) or the field trial differed from the butorphanol + oxygen boma trial (b). The shaded area represents the range of normal values in standing, unrestrained white rhinoceroses (Citino & Bush 2007).

FIGURE 2: Bicarbonate concentration (HCO_3) in immobilised white rhinoceroses.

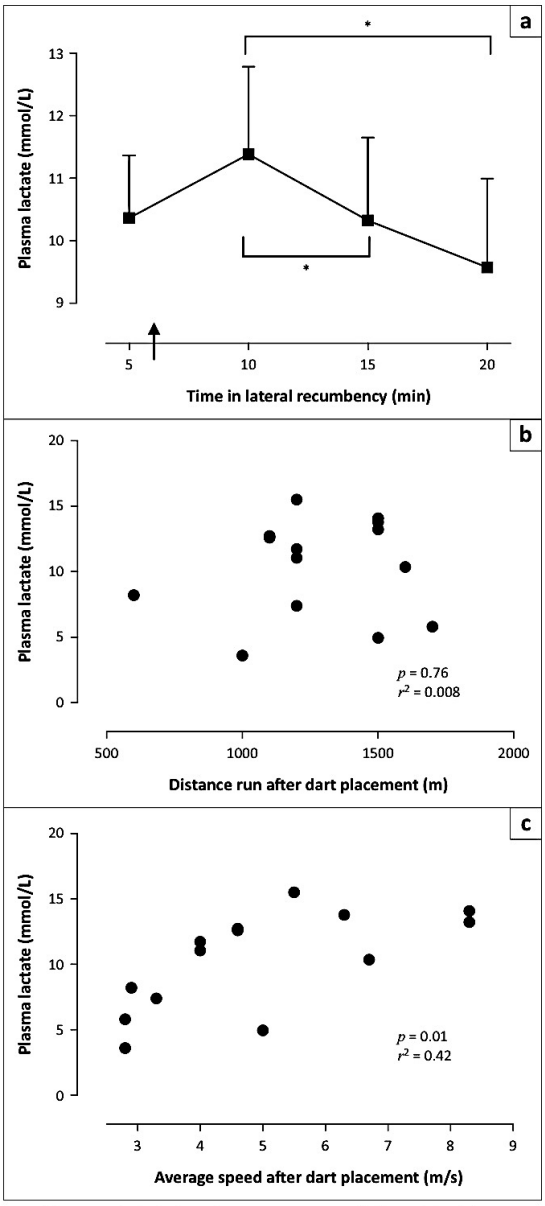
$p < 0.01$, 5 min vs. 10 min). Although heart rate continued to decrease in both boma- and field-immobilised rhinoceroses throughout the immobilisation period, they were still tachycardic (78 beats/min ± 20 beats/min and 70 beats/min ± 9 beats/min in field- and boma-immobilised rhinoceroses respectively) 20 min into the immobilisation period. There was no difference in heart rate between the boma treatment group (butorphanol + oxygen) and field-immobilised rhinoceroses. However, in both the field and boma trials rhinoceroses given butorphanol + oxygen had lower heart rates than those in the boma control trial at 10, 15 and 20 min [$F_{(2,28)} = 22.68$, $p < 0.0001$], in spite of the decrease in heart rate over time in the control animals (139 beats/min ± 9 beats/min at 5 min versus 119 beats/min ± 18 beats/min at 20 min, $p < 0.05$).

Arterial blood pressure

The field-immobilised rhinoceroses were initially hypertensive (mean arterial pressure [MAP] 158 mmHg ± 18 mmHg; normal range for indirect MAP 108 mmHg – 135 mmHg [Citino & Bush 2007], Figure 4b). Following the supportive intervention blood pressure immediately decreased to normotensive levels (MAP 114 mmHg ± 16 mmHg at 10 min, $p < 0.01$, 5 vs 10 min) and all values following butorphanol + oxygen were lower than pre-intervention [$F_{(3,42)} = 42.8$, $p < 0.01$]. There were no arterial blood pressure data from boma-immobilised animals for comparison.

Ethical considerations

This project was approved by the Animal Use and Care Committee of South African National Parks (SANParks) as well as the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance 2012/23/04). This trial was a continuation of a study in which the first phase was conducted in boma-held white rhinoceroses. The ARRIVE guidelines for reporting *in vivo* experiments were adhered to (Kilkenny *et al.* 2010).

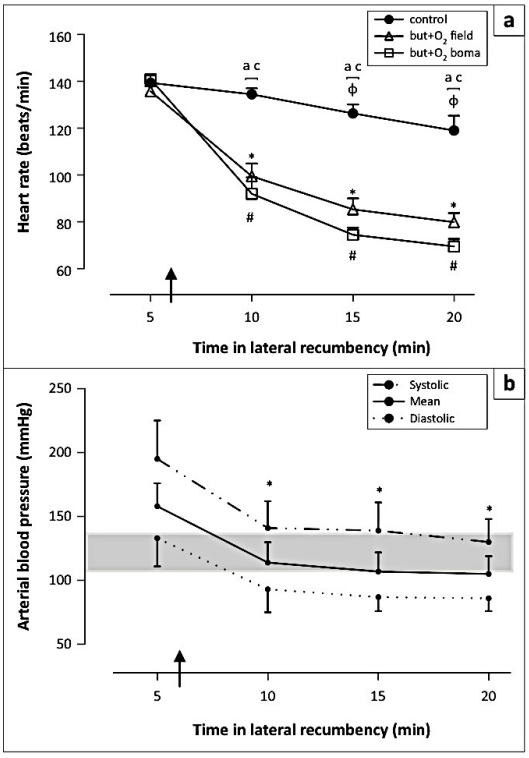


Note: (a) Mean and s.d. of plasma lactate concentration in field-immobilised rhinoceroses given intravenous butorphanol + oxygen insufflation at 6 min (black arrow). The brackets with the asterisk (*) indicate the time points at which the lactate concentrations were different from each other; (b) plasma lactate concentration at 5 min of the 14 field-immobilised white rhinoceroses was correlated with the estimated distance each rhinoceros ran during induction (from dart placement to immobilisation); (c) plasma lactate concentration at 5 min of the 14 field-immobilised white rhinoceroses was correlated with the average speed each rhinoceros ran during induction (from dart placement to immobilisation).

FIGURE 3: (a–c) Plasma lactate.

Discussion

Critical cardiorespiratory abnormalities that occur in chemically immobilised free-ranging white rhinoceroses



Note: (a) Mean and s.d. of the heart rate (beats/min) of field-immobilised rhinoceroses given intravenous butorphanol + oxygen insufflation (but + O_2 field, n = 14), boma-immobilised rhinoceroses given intravenous butorphanol + oxygen insufflation (but + O_2 boma, n = 8) and boma-immobilised rhinoceroses given intravenous sterile water (control, n = 8). The asterisk (*) indicates values in the field trial that differed from pre-intervention values (5 min), whilst # indicates values in the boma treatment trial that differed from pre-intervention values (5 min) and ϕ indicates values in the boma control trial that differed from values at 5 min. The brackets indicate the time points at which the butorphanol + oxygen field trial differed from the control (a) or the butorphanol + oxygen boma trial differed from the control (c); (b) mean and s.d. of arterial blood pressure (mmHg) in field-immobilised rhinoceroses. The black arrow indicates the time at which butorphanol was administered and oxygen insufflation initiated. The asterisk (*) indicates values that differed from pre-intervention values (5 min), whilst the shaded area represents the normal range of the indirect mean arterial pressure in standing, unrestrained white rhinoceroses (Citino & Bush 2007).

FIGURE 4: (a, b) Heart rate and arterial blood pressure.

can be improved significantly with the administration of intravenous butorphanol (a mixed opioid agonist-antagonist) at the beginning of the immobilisation, together with oxygen insufflation throughout the recumbent period. In a previous study of boma-immobilised rhinoceros it was shown that intravenous butorphanol with oxygen insufflation was better than butorphanol alone, oxygen insufflation alone, or a control in which no supportive treatment was administered, in reversing the hypoxaemia associated with opioid-induced immobilisation (Haw *et al.* 2014). Although the effects of immobilising and supportive drugs are probably best assessed in captive conditions where there is less stress and variability, results cannot be directly extrapolated to the field, and follow-up field studies are essential to assess the efficacy of treatments properly.

In the current study the effects of intravenous butorphanol with oxygen insufflation on cardiorespiratory responses of field-immobilised white rhinoceroses were evaluated for the first time and compared with the responses of boma-immobilised white rhinoceroses. The same protocol as that used in the boma study and a similar cohort of animals (sub-adult male white rhinoceroses) were used. The approach allowed investigation of whether a supportive intervention, demonstrated to improve hypoxaemia under tightly controlled conditions in a boma, was equally effective in field-immobilised animals. Compared with the trial in bomas, in the field darting drug doses (based on estimated animal mass) and body condition may vary more between individuals, whilst cardiorespiratory responses are also likely to be altered by increased exertional and psychological stress associated with helicopter darting.

It was found that butorphanol combined with oxygen insufflation indeed also improved oxygenation in field-immobilised white rhinoceroses. However, unlike boma-immobilised rhinoceroses, the hypoxaemia was not fully corrected. On the other hand, the chase before immobilisation in free-ranging rhinoceroses led to a less severe hypopnoea throughout immobilisation compared with the boma-immobilised rhinoceroses. The higher respiratory rate resulted in better ventilation, as demonstrated by the lower P_aCO_2 observed in field-immobilised compared with boma-immobilised rhinoceroses at 5 min. Ventilation further improved following the intervention. As respiratory rate increased only transiently after administration of butorphanol and oxygen, it is likely that an increased tidal volume together with decreased carbon dioxide production – parameters that were not measured in this study – accounted for the continued fall in P_aCO_2 over time.

Despite the lower partial pressure of carbon dioxide in arterial blood, field-immobilised rhinoceroses initially had a more severe acidaemia than their boma-immobilised counterparts, suggesting that metabolic acidosis was a significant contributor to the lower pH (DiBartola 2006). The low pH in the presence of low bicarbonate levels (< 22 mmol/L) also indicated that the acidosis was primarily of metabolic origin, as plasma bicarbonate moves into the cells to buffer the high levels of lactate (Kellum 2007). Although lactate concentrations were not measured in rhinoceroses in the boma trials, the high concentration of plasma lactate (11.4 mmol/L ± 5.2 mmol/L) in the field-immobilised rhinoceroses suggests that lactic acidosis was likely the primary contributor to the acidaemia. However, over time, and following administration of the supportive treatment, the pH increased in field-immobilised rhinoceroses such that by the end of the immobilisation period there was no difference in arterial pH between boma- and field-immobilised rhinoceroses. Plasma lactate concentration decreased from 11.4 ± 5.2 mmol/L at 10 min to 9.6 mmol/L ± 5.0 mmol/L at 20 min. It is difficult to discern whether the decreased lactate concentration reflects rapid metabolism of lactate over time or a response to the treatment intervention. P_aCO_2 in field-immobilised rhinoceroses also decreased from 63.0 mmol/L ± 7.7 mmHg at 5 min to 55.3 mmol/L ± 5.2 mmHg at 20 min. The improvement of pH was therefore probably a

result of the combined effects of lactate metabolism, improved minute ventilation and decreased carbon dioxide production.

In parallel with respiratory depression and acid-base imbalances, immobilisation also resulted in altered cardiovascular function in free-ranging rhinoceroses. At the start of the immobilisation period (5 min), the rhinoceroses were severely tachycardic and moderately hypertensive. Administration of butorphanol and oxygen lowered the heart rate significantly, although normal values were not attained and heart rate remained about twice as high as that expected in standing, unsedated white rhinoceroses (Citino & Bush 2007). However, the initial hypertension completely resolved following administration of butorphanol and oxygen.

Although butorphanol and oxygen completely corrected parameters such as blood pressure, oxygenation was not entirely corrected in the field trial, according to suggested normal values obtained from unrestrained white rhinoceroses in similar environmental conditions (Citino & Bush 2007). However, the peak arterial oxygen concentration observed (81.2 mmol/L ± 23.7 mmHg) was higher than that found in other studies where partial reversal of etorphine and change in body position were used to improve oxygenation (Fahlman 2008; Miller *et al.* 2013; Wenger *et al.* 2007). Moreover, it has been suggested that rhinoceroses may be better able to maintain adequate tissue oxygenation with lower P_aO_2 values than smaller mammals can as a result of the rhinoceros's higher oxygen affinity for haemoglobin (lower P_{50}) and lower tissue metabolic rate (Ball, Larsen & Wagman 2011; Baumann, Mazur & Braunitzer 1984; Heard *et al.* 1992). Therefore the improvement in arterial oxygenation in the rhinoceroses in this study may have been sufficient to prevent hypoxic damage to muscles and other vital organs, thereby reducing the risk of capture-related morbidity such as myopathy, renal failure, abortion and cardiac failure (Heard *et al.* 1992). Although lactate concentrations were high in field-immobilised rhinoceroses, it is believed that these elevated values were mainly as a result of the anaerobic metabolism during the physical exertion before immobilisation, rather than the lack of oxygen supply to cells during immobilisation, as lactate concentration decreased towards the end of the immobilisation period.

It is therefore believed that aerobic metabolism predominated by the end of the immobilisation period in both field- and boma-immobilised rhinoceroses. That P_aO_2 was completely corrected in the boma trial by the administration of butorphanol and oxygen, but not in the field trial, is somewhat surprising, but not entirely unexpected. Etorphine has been implicated in development of pulmonary hypertension (Heard *et al.* 1996; Meyer *et al.* 2015). In addition, activation of the sympathetic nervous system will induce pulmonary hypertension through vasoconstriction, via activation of alpha-1 and alpha-2 adrenoreceptors in the pulmonary vasculature (Hyman & Kadowitz 1986; Kadowitz & Hyman 1973; Salvi 1999). Similarly, hypoxia (Aaronson *et al.* 2006) and acidaemia (Harthoorn & Young 1976) also result in vasoconstriction in the pulmonary vasculature. Moreover, increased sympathetic tone will cause an increase in cardiac output resulting in greater blood flow to the lungs, thus further increasing pulmonary pressure in the vasoconstricted vessels.

It is hypothesised that in the field-immobilised rhinoceroses the psychological and physiological stress response associated with fleeing the helicopter resulted in a higher sympathetic outflow and pulmonary vasoconstriction compared with that in the habituated boma-immobilised rhinoceroses, resulting in more severe pulmonary hypertension. Pulmonary hypertension, leading to pulmonary congestion and possibly oedema (Gao & Raj 2005), will reduce oxygen diffusion from the alveoli into the arterial blood. Increased cardiac output resulting from sympathetic activation and exertion will also increase the speed of blood flow and result in decreased time for red blood cells to traverse alveolar capillaries in the pulmonary bed. If this transit time is less than 0.25 s, oxygen will not have enough time to diffuse and equilibrate in the blood (West 2012). Thus, functional changes in the pulmonary vasculature of field-immobilised white rhinoceroses may account for the differences observed in arterial oxygen concentration between field- and boma-immobilised rhinoceroses after they received identical supportive treatments.

In addition, the difference in arterial oxygen concentration between field- and boma-immobilised rhinoceroses may be attributed to differences in their metabolic rate at immobilisation. Animals with higher metabolism from exertion will have greater tissue oxygen consumption and lower venous oxygen contents, and hence greater partial pressure oxygen gradients between alveolar air and pulmonary arterial blood entering the pulmonary capillaries. In these field-immobilised rhinoceroses, with already compromised oxygen diffusion capabilities as described above, this increased gradient may have reduced the efficacy of the treatment intervention in oxygenating arterial blood. Although metabolism was not measured, it is known that anaerobic metabolism in field-immobilised rhinoceroses was significant, as evidenced by the high lactate values. In another study, where boma-held white rhinoceroses were immobilised with a combination of etorphine, azaperone and hyaluronidase, the highest median lactate value was 2.54 mmol/L (Buss *et al.* 2015), significantly lower than that found in the field-immobilised rhinoceroses (11.52 mmol/L ± 5.08 mmol/L).

Metabolic acidosis (characterised by low arterial pH, high lactate and low bicarbonate concentrations), together with a respiratory acidosis (characterised by low arterial pH and high P_aCO_2) contributed to the overall acidaemia observed in the field-immobilised rhinoceroses. However, metabolic acidosis was most likely the primary contributor to the acidosis as the hypercapnia was relatively mild. In the field-immobilised rhinoceroses both the hypercapnia and acidaemia improved from 5 min (pre-intervention) to 10 min

(4 min after administration of butorphanol and oxygen). Surprisingly, in a previous study where oxygen insufflation was used on its own to improve arterial oxygenation in field-immobilised white rhinoceroses, the initial acidaemia and hypercapnia did not improve (Bush *et al.* 2004). Thus, butorphanol combined with oxygen appears superior to oxygen insufflation alone in field-immobilised white rhinoceroses.

Results from another recent study (Miller *et al.* 2013) indicated that butorphanol may be better administered in the dart mixture rather than after immobilisation has occurred. The authors showed that when butorphanol was administered to the rhinoceros in the immobilising dart mixture, the rhinoceros's plasma lactate concentration was lower and acidaemia less severe compared with when it was administered subsequent to immobilisation. The authors concluded that butorphanol in the dart mixture led to a decreased intensity of exertion during induction, which resulted in less severe metabolic derangements. The finding that plasma lactate concentration was positively correlated with the speed at which the rhinoceros ran after dart placement supports the view that decreased intensity of exertion during induction will decrease the severity of the acidaemia. However, rhinoceroses were still severely hypoxaemic in the study when butorphanol was administered with the dart mixture (Miller *et al.* 2013). Further studies are necessary to determine whether butorphanol administered in the dart, together with oxygen administered during recumbency, would result in improved acid-base balance together with increased oxygenation in immobilised white rhinoceroses.

In addition to arterial blood gas abnormalities, etorphine-induced immobilisation in white rhinoceroses has been known to induce hypertension, as observed in this study (Heard *et al.* 1992; Kock *et al.* 1995; LeBlanc *et al.* 1987). Although no definitive mechanism has been identified, tachycardia, peripheral vasoconstriction, or increased sympathetic tone (as a result of hypoxia, exercise or the drug effect) are suggested factors implicated in etorphine-induced hypertension in both rhinoceroses and equids (Daniel & Ling 1972; Heard *et al.* 1992). Azaperone is therefore routinely used in rhinoceros immobilisation combinations for its sedative effects as well as its alpha-1 antagonistic effect, which blocks vasoconstriction induced by opioids (Burroughs *et al.* 2012).

That the tachycardia and hypertension improved in parallel with the improvement in arterial oxygenation following treatment with butorphanol and oxygen suggests that the hypoxia-induced sympathetic response was a predominant factor implicated in the initial hypertension and tachycardia (Schultz, Li & Ding 2007). However, a direct drug effect may also account for the observed changes. Intravenous administration of butorphanol in game-ranched etorphine-immobilised white rhinoceroses has been shown to decrease heart rate and systemic blood pressure (Boardman *et al.* 2014). Similarly, in free-ranging white rhinoceroses, intravenous butorphanol resulted in a decrease in heart rate in etorphine-immobilised white rhinoceros (Miller *et al.* 2013).

Surprisingly, in a study where oxygen insufflation was used to correct etorphine-induced hypoxaemia in the immobilised white rhinoceros, heart rate and blood pressure did not improve with administration of oxygen, despite the increase in arterial oxygenation (Bush *et al.* 2004). It is therefore suggested that butorphanol's significant activity at opioid receptors (Commisky *et al.* 2005) may have a greater effect on the cardiovascular system than the improvement in arterial oxygen concentration alone. It cannot, however, be discounted that some of the observed cardiovascular effects may have been attributed to the azaperone in the dart mixture, which has a longer onset of action than etorphine (Portas 2004), with a peak effect occurring one hour after administration (ZooPharm 2013).

Mortalities of white rhinoceros under etorphine immobilisation have been reported, with worsening hypoxaemia noted as a risk factor for mortality (Kock *et al.* 1995). Therefore the protocol of administering intravenous butorphanol together with oxygen via nasotracheal intubation, which significantly improved oxygenation and reduced hypercapnia, acidaemia and heart rate, would undoubtedly reduce the risk of immobilisation-related morbidity and mortality in field-immobilised white rhinoceroses. To further reduce this risk it is believed that refinement of butorphanol doses, oxygen flow rates and administration methods should be considered.

In addition, further research is needed to clarify which physiological disturbances cause worse respiratory compromise and hypoxia in chased compared to non-chased animals after treatment. Once these pathophysiological effects have been clarified, alternative treatment options aimed at correcting the specific physiological imbalances can be implemented.

Conclusion

The efficacy of oxygen insufflation combined with intravenous butorphanol, administered at the beginning of the immobilisation period, in treating cardiorespiratory compromise in field-immobilised white rhinoceroses was evaluated. This treatment protocol was based on the findings of a previous boma study, where butorphanol and oxygen completely corrected the immobilisation-induced hypoxaemia.

Despite the additional physiological imbalances that occur during field-immobilisations, this treatment intervention was still superior to previous protocols aimed at correcting immobilisation-induced hypoxaemia. Administering oxygen with intravenous butorphanol is a simple, readily available technique that will significantly improve oxygenation, thereby improving the safety of white rhinoceros immobilisation.

Acknowledgements

We thank the staff of the SANParks Veterinary Wildlife Services for dedicated support and assistance with experimental procedures. In particular we thank Marius Kruger for managing the capture team and Jennifer Hofmeyr, Nomkhosi Mathebula and Guy Hausler for field and laboratory assistance. We also thank the South African National Research Foundation, the International Rhino Foundation, Disney's Animal Programs and Environmental Initiatives, SANParks, and the University of the Witwatersrand for financial support.

Competing interests

The authors declare that they have no financial or personal relationships which may have inappropriately influenced them in writing this article.

Authors' contributions

A.F. (University of the Witwatersrand), A.H. (University of the Witwatersrand), G.F. (Disney's Animal Programs and Environmental Initiatives), L.M. (University of Pretoria), M.H. (SANParks), M.M. (Stellenbosch University) and P.B. (SANParks) were all responsible for the study conception and design. The data collection and experimental procedures were compiled by A.F., A.H., G.F., L.M., M.H., M.M. and P.B. Data analysis was dealt with by A.H., A.F. and L.M., and A.H. was responsible for the first draft. Comments on the draft and final approval were completed by A.F., A.H., L.M., M.H., M.M. and P.B.

References

Aaronson, P.I., Robertson, T.P., Knock, G.A., Becker, S., Lewis, T.H., Snetkov, V. *et al.*, 2006, 'Hypoxic pulmonary vasoconstriction: Mechanisms and controversies', *Journal of Physiology* 570, 53–58. http://dx.doi.org/10.1113/jphysiol.2005.098855

Ball, R.L., Larsen, R. & Wagman, J., 2011, 'Investigating the hemoglobin of white rhinoceros (*Ceratotherium simum simum)* and possible implications for anesthesia', in *Proceedings of the 2011 International Elephant and Rhino Conservation and Research Symposium*, Rotterdam, the Netherlands, October 10–14, 2011, pp. 1497–1521.

Baumann, R., Mazur, G. & Braunitzer, G., 1984, 'Oxygen binding properties of hemoglobin from the white rhinoceros (β_2-GLU½) and the tapir', *Respiration Physiology* 56, 1–9. http://dx.doi.org/10.1016/0034-5687(84)90124-5

Boardman, W.S.J., Caraguel, C.G.B., Raath, J.P. & Van Zijll Langhout, M., 2014, 'Intravenous butorphanol improves cardiopulmonary parameters in game-ranched white rhinoceroses (*Ceratotherium simum*) immobilized with etorphine and azaperone', *Journal of Wildlife Diseases* 50, 849–857. http://dx.doi.org/10.7589/2013-12-327

Burroughs, R., Meltzer, D. & Morkel, P., 2012, 'Applied pharmacology', in M.D. Kock, D. Meltzer & R. Burroughs (eds.), *Chemical and physical restraint of wild animals,* 2nd edn., pp. 53–80, IWVS (Africa), Greyton.

Bush, M., Raath, J.P., Grobler, D. & Klein, L., 2004, 'Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen', *Journal of the South African Veterinary Association* 72, 79–84. http://dx.doi.org/10.4102/jsava.v75i2.457

Buss, P., Olea-Popelka, F., Meyer, L., Hofmeyr, J., Mathebula, N., Kruger, M., Brüns, A. *et al.*, 2015, 'Evaluation of cardiorespiratory, blood gas, and lactate values during extended immobilization of white rhinoceros (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 46, 224–233. http://dx.doi.org/10.1638/2014-0089R.1

Citino, S.B. & Bush, M., 2007, 'Reference cardiopulmonary physiologic parameters for standing, unrestrained white rhinoceroses (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 38, 375–379. http://dx.doi.org/10.1638/2006-0007R1.1

Commisky, S., Fan, L-W., Ho, I.K. & Rockhold, R.W., 2005, 'Butorphanol: Effects of a prototypical agonist-antagonist analgesic on κ-opioid receptors', *Journal of Pharmacological Sciences* 98, 109–116. http://dx.doi.org/10.1254/jphs.CRJ05001X

Cornick-Seahorn, J.L., Mikota, S.K., Schaeffer, D.O., Ranglack, G.S. & Boatright, S.B., 1995, 'Isoflurane anesthesia in a rhinoceros', *Journal of the American Veterinary Medical Association* 206, 508–511.

Daniel, M. & Ling, C.M., 1972, 'The effect of an etorphine-acepromazine mixture on the heart rate and blood pressure of the horse', *Veterinary Record* 90, 336–339. http://dx.doi.org/10.1136/vr.90.12.336

DiBartola, S.P., 2006, 'Metabolic acid-base disorders', in S.P. DiBartola (ed.), *Fluid, electrolyte and acid-Base disorders in small animal practice,* 3rd edn., pp. 251–283, Saunders Elsevier, St Louis. http://dx.doi.org/10.1016/B0-72-163949-6/50013-8

Fahlman, A., 2008, 'Advances in wildlife immobilisation and anaesthesia: Clinical and physiological evaluation in selected species', PhD thesis, Swedish University of Agricultural Sciences.

Fahlman, A., Foggin, C. & Nyman, G., 2004, 'Pulmonary gas exchange and acid-base status in immobilized black rhinoceros (*Diceros bicornis*) and white rhinoceros (*Ceratotherium simum*) in Zimbabwe', *Proceedings of the joint conference of the American Association of Zoo Veterinarians, American Association of Wildlife Veterinarians and the Wildlife Disease Association*, 2004, pp. 523–525.

Gao, Y. & Raj, U., 2005, 'Role of veins in regulation of pulmonary circulation', *American Journal of Physiology - Lung Cellular and Molecular Physiology* 288, L213–L226. http://dx.doi.org/10.1152/ajplung.00103.2004

Harthoorn, A.M. & Young, E., 1976, 'Pulmonary hypertension in relationship to acidaemia after maximum forced exercise. A further preliminary report', *Journal of the South African Veterinary Association* 47, 187–189.

Hattingh, J. & Knox, C.M., 1994, 'Arterial blood pressure and blood gas composition of white rhinoceroses under etorphine anaesthesia', *South African Journal of Wildlife Research* 24, 12–14.

Haw, A., Hofmeyr, M., Fuller, A., Buss, P., Miller, M., Fleming, G. *et al.*, 2014, 'Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*)', *BMC Veterinary Research* 10, Art.#253. http://dx.doi.org/10.1186/s12917-014-0253-0

Heard, D.J., Nichols, W.W., Buss, D. & Kollias, G.V., 1996, 'Comparative cardiopulmonary effects of intramuscularly administered etorphine and carfentanil in goats', *American Journal of Veterinary Research* 57, 87–96.

Heard, D.J., Olsen, J.H. & Stover, J., 1992, 'Cardiopulmonary changes associated with chemical immobilisation and recumbency in a white rhinoceros (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 23, 197–200.

Hyman, A. & Kadowitz, P., 1986, 'Enhancement of α- and β- adrenoceptor responses by elevations in vascular tone in pulmonary circulation', *American Journal of Physiology* 250, H1109–H1116.

Kadowitz, P. & Hyman, A., 1973, 'Effect of sympathetic nerve stimulation on pulmonary vascular resistance in the dog', *Circulation Research* 32, 221–227. http://dx.doi.org/10.1161/01.RES.32.2.221

Kellum, J.A., 2007, 'Disorders of acid-base balance', *Critical Care Medicine* 35, 2630–2636. http://dx.doi.org/10.1097/01.CCM.0000286399.21008.64

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. & Altman, D.G., 2010, 'Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research', *PLoS Biology* 8(6), e1000412. http://dx.doi.org/10.1371/journal.pbio.1000412

Kock, M.D., Morkel, P., Atkinson, M. & Foggin, C., 1995, 'Chemical immobilisation of free-ranging white rhinoceros (*Ceratotherium simum simum*) in Hwange and Matobo National Parks, Zimbabwe, using combinations of etorphine (M99), fentanyl, xylazine and detomidine', *Journal of Zoo and Wildlife Medicine* 26, 207–219.

LeBlanc, P.H., Eicker, S.W., Curtis, M. & Beehler, B., 1987, 'Hypertension following etorphine anesthesia in a rhinoceros (*Diceros simus*)', *Journal of Zoo Animal Medicine* 18, 141–143. http://dx.doi.org/10.2307/20094825

Meyer, L.C.R., Hetem, R., Mitchell, D. & Fuller, A., 2015, 'Hypoxia following etorphine administration in goats (*Capra hircus*) results more from pulmonary hypertension than from hypoventilation', *BMC Veterinary Research* 11, Art.#18. http://dx.doi.org/10.1186/s12917-015-0337-5

Miller, M., Buss, P., Joubert, J., Mathebula, N., Kruger, M., Martin, L. *et al.*, 2013, 'Use of butorphanol during immobilisation of free-ranging white rhinoceros (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 44, 55–61. http://dx.doi.org/10.1638/1042-7260-44.1.55

Portas, T.J., 2004, 'A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species', *Australian Veterinary Journal* 82, 542–549. http://dx.doi.org/10.1111/j.1751-0813.2004.tb11196.x

Raath, J.P., 1999, 'Anesthesia of white rhinoceros', in M.E. Fowler & R.E. Miller (eds.), *Zoo and wild animal medicine, current therapy,* 4th edn., pp. 556–571, W.B. Saunders, Philadelphia.

Salvi, S.S., 1999, 'α_1-Adrenergic hypothesis for pulmonary hypertension', *Chest* 115, 1708–1719. http://dx.doi.org/10.1378/chest.115.6.1708

Schultz, H.D., Li, Y.L. & Ding, Y., 2007, 'Arterial chemoreceptors and sympathetic nerve activity. Implications for hypertension and heart failure', *Hypertension* 50, 6–13. http://dx.doi.org/10.1161/HYPERTENSIONAHA.106.076083

Wenger, S., Boardman, W., Buss, P., Govender, D. & Foggin, C., 2007, 'The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field-anaesthetized white rhinoceroses (*Ceratotherium simum*)', *Journal of Zoo and Wildlife Medicine* 38, 380–387. http://dx.doi.org/10.1638/2006-0038R.1

West, J.B., 2012, *Respiratory physiology the essentials,* 9th edn., Lippincott William & Wilkins, Baltimore.

ZooPharm, 2013, *Azaperonetartrate_websiteinfosheet_11_2013*, viewed 21 April 2015, from http://www.wildpharm.com/azaperone-tartrate

CHAPTER 5

Ampakine CX1942 attenuates opioid-induced respiratory depression and corrects hypoxaemia in etorphine-immobilized goats (*Capra hircus*)

Haw AJ, Meyer LCR, Greer JJ and Fuller A

***Veterinary Anaesthesia and Analgesia*, in press**

(Awaiting proof from journal)

ABSTRACT

Objectives To determine whether CX1942 reverses respiratory depression in etorphine-immobilized goats, and to compare its effects to those of doxapram hydrochloride.

Study design Prospective, crossover experimental trial conducted at 1753 m.a.s.l.

Animals Eight adult female Boer goats (*Capra hircus*) with a mass of 27.1 ± 1.6 kg (mean ± SD).

Methods Following immobilization with 0.1 mg kg^{-1} etorphine, goats received doxapram, CX1942 or sterile water intravenously, in random order in three trials. Respiratory rate, ventilation and tidal volume were measured continuously. An arterial blood sample, for determination of PaO_2, $PaCO_2$, pH and SaO_2, was taken 2 minutes before and then at each 5-minute interval after drug administration for 25 minutes.

Results Doxapram corrected etorphine-induced respiratory depression but also led to arousal and hyperventilation 2 minutes after its administration, as indicated by the low $PaCO_2$ (27.8 ± 4.5 mmHg) and ventilation of 5.32 ± 5.24 L $minute^{-1}$ above pre-immobilization values. CX1942 improved the respiratory parameters and corrected etorphine's hypoxaemic effects more gradually than did doxapram, with a more sustained improvement in PaO_2 and SaO_2 when compared to the control trial.

Conclusions CX1942 attenuated opioid-induced respiratory depression and corrected the hypoxaemic effects of etorphine in immobilized goats.

Clinical relevance Ampakines potentially offer advantages over doxapram, a conventional treatment, in reversing etorphine-induced respiratory depression without causing unwanted side-effects, particularly arousal, in immobilized animals.

Keywords CX1942, doxapram, hyperventilation, wildlife

INTRODUCTION

For more than 50 years, etorphine's advantageous pharmacodynamics has enabled veterinarians to immobilize free-ranging wild animals for management and veterinary interventions. However, opioid-induced respiratory depression remains a significant, and potentially life-threatening, side-effect of chemical immobilization. Opioid-induced respiratory depression is mediated mainly via direct activation of mu-opioid receptors, which are abundantly expressed in brainstem respiratory control centres (Pattinson 2008; Dahan et al. 2010). Opioids also depress the hypoxic ventilatory response and hypercapnic ventilatory response through depression of central chemoreception (Pattinson 2008).

It is also through activation of mu-opioid receptors that etorphine's desirable actions, such as analgesia, sedation and catatonia, are produced (Dahan et al. 2010). Thus opioid-antagonists such as naltrexone and naloxone, which are effective at counteracting opioid-induced respiratory depression, will also antagonize analgesia, sedation and catatonia, which is an undesirable outcome when etorphine is used as a chemical immobilizing agent in wildlife.

Doxapram, an analeptic agent, often is used to stimulate respiration in opioid-immobilized wild animals (Burroughs et al. 2012). Although respiration is significantly improved following the administration of doxapram, the effects are short-lived and general central nervous system stimulation can result in undesirable side-effects (Yost 2006).

More recently, a novel approach using ampakines has been developed for alleviating respiratory depression caused by disease and drug administration (Ren et al. 2006; Ren et al. 2009; Ren et al. 2012; Ren et al. 2013; Ren et al. 2015; El Mallah et al. 2015). Ampakines are a structurally diverse family of small molecules that readily cross the blood-brain barrier and bind to an allosteric site on the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor to enhance fast, excitatory transmission (Lynch 2006). AMPA receptor-mediated conductances are important contributors to respiratory rhythmogenesis and inspiratory drive to cranial and spinal motoneurons (Greer et al. 1991; Funk et al. 1993; Pace et al. 2007). Indeed, ampakines suitable for oral administration have been shown to counteract opioid-induced respiratory depression without affecting mu-opioid analgesia, in both rats and humans (Ren et al. 2006; Ren et al. 2009; Oertel et

al. 2010). However, whether an injectable ampakine formulation will reverse respiratory depression, without affecting the immobilizing properties of etorphine, is not yet known.

Here, we used the goat (*Capra hircus*), a domestic artiodactyl, as a model to evaluate the effectiveness of an ampakine, CX1942, in attenuating opioid-induced respiratory depression in etorphine-immobilized ungulates. CX1942 is a novel water-soluble low-impact ampakine that is rapidly absorbed and pharmacologically active (J. Greer, personal communication). This model enabled us to evaluate CX1942 without the background of a severe stress response that normally accompanies the capture of wild animals. We aimed to compare the effects of CX1942 to those of the known respiratory stimulant doxapram, with the hypothesis that CX1942 would be more effective than doxapram in attenuating etorphine-induced respiratory depression.

MATERIALS AND METHODS

This project was approved by the University of the Witwatersrand Animal Ethics Screening Committee (clearance 2013/26/04) and the ARRIVE guidelines for reporting *in vivo* experiments were adhered to (Kilkenny et al. 2010). We used eight healthy adult female Boer goats (*Capra hircus*) with body mass 27.1 ± 1.6 kg (mean ± SD). A sample size of eight was chosen based on the statistically significant and clinically relevant results from a previous controlled repeated measures study where eight animals were also used (Meyer et. al. 2006). The goats were housed together in temperature-controlled indoor pens in the Central Animal Services, University of the Witwatersrand, South Africa, at an altitude of 1753 meters, on a 12 hour light/dark cycle (lights on at 06:00). Air temperature ranged between 21°C and 23°C and relative humidity between 23% and 47%. The goats had access to water *ad libitum* and were fed hay and sheep concentrate pellets. Over a period of six weeks, before the start of data collection, each goat was habituated to being restrained in sternal recumbency on a work table with a face mask over its muzzle.

All measurements were made indoors between 08:00 and 13:30. Barometric pressure, measured by the on-board barometer of the blood-gas analyser, ranged from 622.4 to 626.2 mmHg (83.0–83.5 kPa); the partial pressure of inspired oxygen therefore was about 131 mmHg (17.5 kPa), about 30 mmHg lower than the partial pressure of inspired oxygen at sea level.

Experimental procedures:

The experiment consisted of three trials in which each goat was given etorphine + sterile injectable water (control), etorphine + doxapram, and etorphine + CX1942, in a random order at two-weekly intervals. Trial randomization was determined using a computer generated random number sequence. Etorphine hydrochloride (Captivon 9.8 mg mL^{-1}; Wildlife Pharmaceuticals Pty Ltd., South Africa) was injected intramuscularly into the right gluteal muscle (time 0) at a dose of 0.1 mg kg^{-1}. This dose adequately immobilized and sedated the goats for the duration of the 30 minute experimental period. Doxapram hydrochloride (Dopram-Fresenius 20 mg mL^{-1}; Bodene Pty Ltd., South Africa) was injected intravenously (IV) at a dose of 1 mg kg^{-1}. CX1942 (Cortex Pharmaceuticals, NJ, USA) was made up to a concentration of 50 mg mL^{-1} by dissolving the solute in sterile physiological saline (0.9% NaCl, Fresenius Kabi Manufacturing, South Africa), and was injected IV at a dose of 20 mg kg^{-1}. This dose was established in a pilot dose-response study, where a higher dose was not more effective, and a lower dose did not produce a consistent response. In the control trial, 2 mL of sterile injectable water (Water for Injection, Kyron Laboratories, South Africa) was injected IV. Doxapram, CX1942 and sterile water (control) were each injected at 8 minutes after the etorphine during the respective trials.

The goats were weighed before each trial and were starved for at least 12 hours before the trial to reduce the risk of bloat and regurgitation of ingesta. In each trial, the goat was brought from its pen to the laboratory and placed on a table in sternal recumbency. A handler held the horns and positioned the goat's head so that the neck was aligned with the spinal column and the head was elevated above the thorax with the nose pointing downwards.

Both ears were shaved and a topical local anaesthetic, amethocaine hydrochloride (Anethaine Cream, Winthrop Pharmaceuticals Pty Ltd., South Africa), was applied. The ears were then swabbed with 5 % chlorhexidine gluconate (Hibitane, Astra Zeneca, South Africa) in 100 % ethanol. A 22 gauge catheter (Introcan, B Braun, Melsungen AG, Germany) was placed in one of the auricular arteries and connected to a 3-way stopcock valve (Sabex Manufacturing (Pty) Ltd. South Africa), which was secured to the ear with

adhesive tape (Leukoplast, Germany). A second 22-gauge catheter was placed in an auricular vein in the opposite ear to facilitate intravenous drug administration.

A clear canine anaesthetic face mask (J-298C, Jorgensen Laboratories, CO, USA) was placed over the nose and mouth of the goat. A tight seal between the goat's muzzle and the face mask was achieved by using a modified latex glove. The face mask was connected to a two-way valve which directed all the expired air into the PowerLab Exercise Physiology System (ADInstruments Pty Ltd, NSW, Australia), via a respiratory flow head (MLT300L, ADInstruments Pty Ltd) linked to a spirometer (ML141, ADInstruments Pty Ltd) and a gas mixing chamber (MLA245, ADInstruments Pty Ltd), in which expired gas temperature was measured by a thermistor pod (ML309, ADInstruments Pty Ltd). The data from these modules were collected via the PowerLab 8/30 amplifier (ML870, ADInstruments Pty Ltd) and integrated with the Metabolic Module software (ADInstruments Pty Ltd) to measure minute ventilation, tidal volume and respiratory rate. Before each set of measurements the spirometer was zeroed and calibrated using a 3L calibration syringe.

Naltrexone hydrochloride (Trexonil 50 mg kg^{-1}, Wildlife Pharmaceuticals Pty Ltd., South Africa) was administered intravenously 28 minutes after the etorphine injection at a dose of 2 mg kg^{-1}, which adequately reversed the effects of etorphine within one minute of administration. Data were recorded for 3 minutes before the injection of etorphine until one minute after the administration of naltrexone. A 0.5 mL arterial blood sample was drawn 2 minutes before etorphine injection and at 5, 10, 15, 20 and 25 minutes thereafter. The catheter was flushed with 2 mL heparinized saline after each sample. Arterial pH, partial pressures of oxygen (PaO_2), and carbon dioxide ($PaCO_2$), and haemoglobin oxygen saturation (SaO_2) were measured immediately after sample collection using a pre-calibrated blood gas analyser with pre-calibrated blood gas cassettes (Roche OPTI CCA Analyzer + OPTI cassette B, Kat Medical (Pty) Ltd, Johannesburg, South Africa). Partial pressures were measured and reported at 37°C. We considered the goats to have etorphine-induced hypoxaemic effects when their PaO_2 values were significantly lower than those before immobilization. We did not use a standardized cut-off value (e.g. < 60 mmHg) to define hypoxaemia, as the altitude at which our study was conducted had an effect on the partial pressures of arterial gases of the goats.

The quality of immobilization was scored one minute after each arterial blood sample. The score ranged from 1 (fully awake) to 5 (life-threatening immobilization) (Table 1).

Data analysis

We used GraphPad Prism version 6.00 for Mac OS X (GraphPad Software Inc., CA, USA) for statistical analyses. All results are reported as mean ± SD, and $p < 0.05$ was considered statistically significant. For all the variables measured by the blood gas analyser and PowerLab Exercise Physiology System, a two-way repeated measures analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used to test for differences between responses to doxapram hydrochloride, CX1942 and sterile water (control) at -2, 5, 10, 15, 20 and 25 minutes, and between time points for each intervention. The immobilization scores were not statistically compared.

RESULTS

Respiratory rate (Figure 1A):

The respiratory rate of the goats during the three trials changed over time ($F_{(5,105)} = 49.51$, $p < 0.0001$), with differences also observed between the trials ($F_{(2,21)} = 3.84$, $p = 0.04$). Before etorphine was injected (-2 minutes), respiratory rates were similar across trials. Administration of etorphine resulted in hypopnea at 5 minutes in all three trials. The goats' respiratory rates did not improve after the injection of sterile water (control) or CX1942 at any timepoint. Doxapram administration, however, resulted in an increased respiratory rate at 10, 15 and 25 minutes. However, the respiratory rates did not return to baseline values.

Tidal volume (Figure 1B):

In all trials, including the control, tidal volumes of the goats at 10 minutes were higher than pre-immobilization values (-2 minutes; $p < 0.05$), and the values at 5 minutes and thereafter did not differ from each other. At all the time points there were no differences between the trials ($F_{(2,21)} = 0.65$, $p = 0.53$). However, tidal volume in goats in the CX1942 trial increased such that it was higher at 10, 15, 20 and 25 minutes compared to before immobilization (-2 minutes; $p < 0.05$).

Change in ventilation (Figure 1C):

Following etorphine administration (5 minutes), ventilation decreased in all groups. The administration of doxapram and CX1942 improved ventilation compared to the control ($F_{(2,21)}$ = 8.74; p = 0.002). Two minutes after doxapram administration, ventilation increased to 5.32 ± 5.24 L minute^{-1} above pre-immobilization levels ($p < 0.0001$, -2 vs. 10 minutes). In one goat, ventilation increased to more than 16 L minute^{-1} above pre-immobilization levels after doxapram, which contributed to the large standard deviation at 10 minutes. Shortly after this hyperventilatory response, ventilation in the goats that had received doxapram decreased to pre-immobilization levels at 15, 20 and 25 minutes. Compared to when the goats received the control, ventilation was higher after doxapram at 10, 15 and 20 minutes, but not 25 minutes. CX1942 led to a gradual increase in ventilation such that ventilation at 15, 20 and 25 minutes was higher ($p < 0.05$) than at 5 minutes and no different to pre-immobilization levels. Also, the goats in the CX1942 trial had higher ventilation rates at 15, 20 and 25 minutes compared to when they received the control ($p < 0.05$).

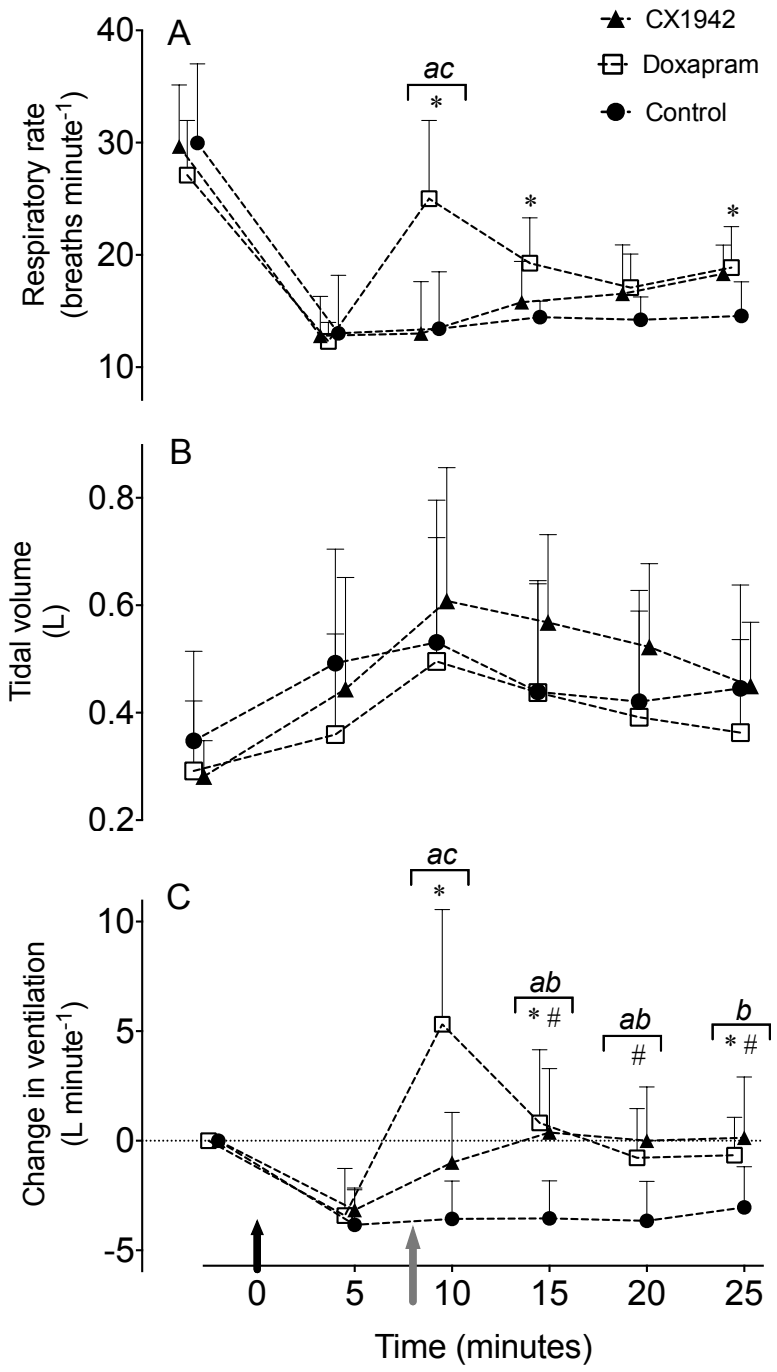


Figure 1. Effects of doxapram (1 mg kg^{-1}), CX1942 (20 mg kg^{-1}) and sterile water (control) injected intravenously (time = 8 minutes, illustrated by the grey arrow) in etorphine-immobilized goats on respiratory rate (A), tidal volume (B) and change in ventilation (C) (mean ± SD, n = 8). The black arrow (time = 0) shows when etorphine (0.1 mg kg^{-1}) was administered intramuscularly. The symbols * and # indicate when values in the doxapram and CX1942 trials respectively, differ from those at 5 minutes. Differences between the treatment trials are indicated by the letters *a* (doxapram *versus* control), *b* (CX1942 *versus* control) and *c* (doxapram *versus* CX1942) (repeated measures two-way ANOVA).

Arterial blood gases (Figure 2):

PaO_2 in the goats changed over time ($F_{(5,105)} = 51.82$, $p < 0.0001$) and between treatments ($F_{(2,21)} = 8.2$, $p = 0.002$). Five minutes after the administration of etorphine, PaO_2 had decreased in all groups. When the goats did not receive any treatment intervention (control), PaO_2 gradually improved but the hypoxaemic effects of etorphine were not corrected at any time during the immobilization period. Doxapram administration resulted in an immediate increase in PaO_2; at 10 minutes PaO_2 was higher than before the intervention ($p < 0.0001$) and higher than the subsequent time points ($p < 0.0001$) but this value was not sustained and decreased to below pre-immobilization levels at 20 minutes and were comparable to the control values. CX1942 administered to immobilized goats, however, resulted in a sustained improvement in PaO_2 compared to the control trial and corrected etorphine's hypoxaemic effects in the goats ($p > 0.05$).

Similarly, etorphine, doxapram and CX1942, led to changes in the arterial haemoglobin oxygen saturation values ($F_{(5,123)} = 29.11$, $p < 0.0001$) that paralleled the changes observed in the PaO_2 results (see Table 2).

As expected, the etorphine-induced hypopnea led to a significant increase ($F_{(5,105)} = 40$, $p < 0.0001$) in $PaCO_2$ at 5 minutes. When the goats did not receive any treatment (control), $PaCO_2$ remained elevated throughout the immobilization period with no difference ($p > 0.05$) in $PaCO_2$ between 25 minutes and 5 minutes. Doxapram administration resulted in an immediate and significant reduction in $PaCO_2$ at 10 minutes, below that of – 2 minutes ($p < 0.01$), but then was similar to that at -2 minutes by 15, 20 and 25 minutes ($p > 0.05$). CX1942 also resulted in a decrease in $PaCO_2$ such that $PaCO_2$ at all the samples after CX1942 administration was lower than that at 5 minutes ($p < 0.05$). The decrease in $PaCO_2$ following CX1942 administration, however, was more gradual than and not as exaggerated as that observed following doxapram administration.

In parallel with the elevated $PaCO_2$ in the goats following etorphine administration, the pH decreased in all trials ($F_{(5,150)} = 20$, $p < 0.0001$). When the goats did not receive any treatment intervention, the pH remained below pre-immobilization values ($p < 0.01$) throughout the immobilization period. Intravenous doxapram resulted in an immediate increase in pH at 10 minutes. Indeed, doxapram administration resulted in a higher pH compared to the control at all time points following the intervention ($p < 0.01$) and the pH

at 15, 20 and 25 minutes was no different to that at -2 minutes ($p > 0.05$). CX1942 administration also led to a rise in pH, but the increase was more gradual, compared to that following doxapram, with values at 15, 20 and 25 minutes higher than at 5 minutes ($p < 0.01$), and no different to pre-immobilization values ($p > 0.05$). CX1942 did not increase pH compared to sterile water (control) except at 15 minutes ($p < 0.05$).

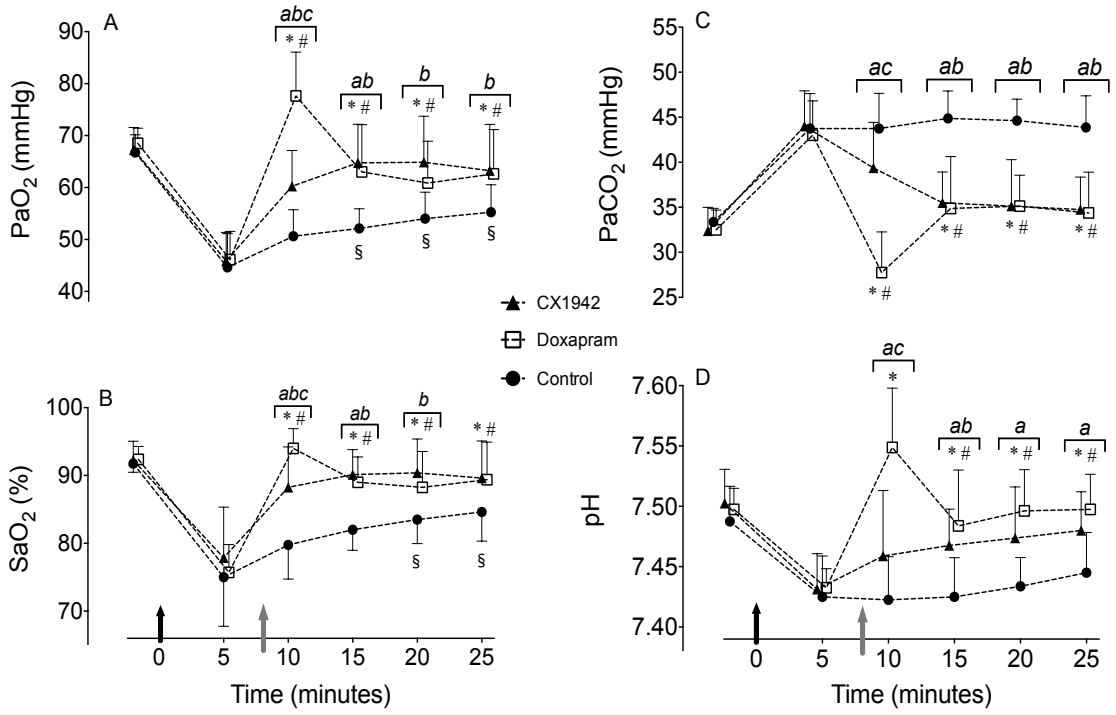


Figure 2. Effects of doxapram (1 mg kg^{-1}), CX1942 (20 mg kg^{-1}) and sterile water (control) injected intravenously (time = 8 minutes, illustrated by the grey arrow) in etorphine-immobilized goats on partial pressure of arterial oxygen (A), haemoglobin oxygen saturation (B), partial pressure of arterial carbon dioxide (C) and arterial pH (D) (mean ± SD, n = 8). The black arrow (time = 0) shows when etorphine (0.1 mg kg^{-1}) was administered intramuscularly. The symbols *, # and § indicate when values in the doxapram, CX1942 and control trials respectively, differ from those at 5 minutes. Differences between the treatment trials at specific time points are indicated by the letters *a* (doxapram vs. control), *b* (CX1942 vs. control) and *c* (doxapram vs. CX1942) (repeated measures two-way ANOVA).

Movement

Following the administration of etorphine, goats in the control and doxapram trials had a median immobilization score of 4 (see Table 1) while the median immobilization score in the CX1942 trial was 3. The immobilization (score 4) was maintained throughout the experimental procedure in the control trial. Doxapram administration, however, resulted in a decrease in the level of immobilization with median scores of 2 (light plane of immobilization with occasional body movement) at 10, 15 and 20 minutes. On the other hand, the administration of CX1942 did not alter the immobilization on a gross level, with a consistent median immobilization score of 3 throughout the experimental period.

Table 1: Description used to score the level of immobilization in goats

Score	**Description**
1	Fully awake
2	Light plane of immobilization, palpebral and ear flick reflexes still present. Movement of one or more body parts (tail, ear, head, limb)
3	Appropriately immobilized with reduced but present palpebral and ear flick reflexes. Occasional movement of one body part.
4	Appropriately immobilized with absent palpebral and ear flick reflexes. No movement of any body parts.
5	Immobilized to a life-threatening level. No response to excessive stimulation.

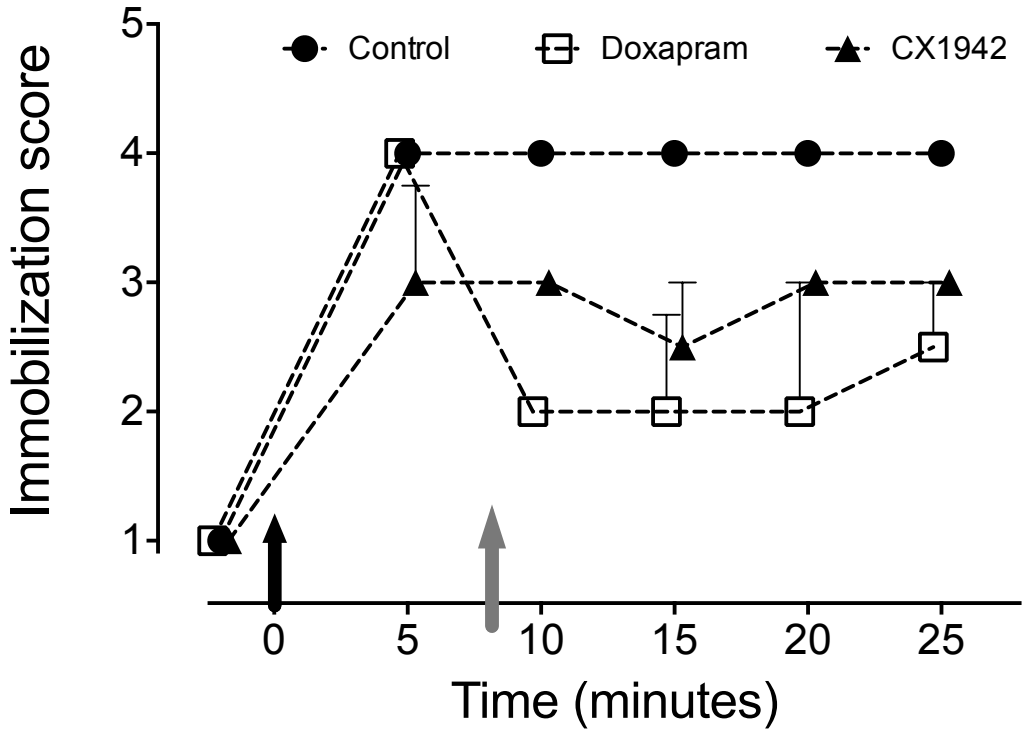


Figure 3. Effects of doxapram (1 mg kg^{-1}), CX1942 (20 mg kg^{-1}) and sterile water (control) injected intravenously (time = 8 minutes, illustrated by the grey arrow) in etorphine-immobilized goats on the immobilization score (Table 1) (median ± interquartile range, n = 8). The black arrow (time = 0) shows when etorphine (0.1 mg kg^{-1}) was administered intramuscularly.

		Timepoint (minutes)					
Variable	**Group**	-2	5	10	15	20	25
Respiratory rate (breaths minute $^{-1}$)	Control	30 ± 7	13 ± 5*	13 ± 5*	14 ± 1*	14 ± 2*	15 ± 3*
	Doxapram	27 ± 5	12 ± 2*	25 ± 7†	19 ± 4*†	17 ± 3*	19 ± 4*†
	CX1942	30 ± 5	13 ± 4*	13 ± 5*	16 ± 4*	17 ± 4*	18 ± 3*
Tidal Volume (L)	Control	0.35 ± 0.17	0.49 ± 0.21	0.53 ± 0.26*	0.44 ± 0.20	0.42 ± 0.21	0.45 ± 0.19
	Doxapram	0.29 ± 0.13	0.36 ± 0.19	0.50 ± 0.23*	0.44 ± 0.21	0.39 ± 0.20	0.36 ± 0.17
	CX1942	0.28 ± 0.07	0.44 ± 0.21	0.61 ± 0.25*	0.57 ± 0.16*	0.52 ± 0.15*	0.45 ± 0.12*
Ventilation (L minute^{-1})	Control	9.82 ± 4.62	5.98 ± 3.14*	6.25 ± 3.10*	6.27 ± 2.96*	6.16 ± 2.92*	6.78 ± 3.30*
	Doxapram	7.82 ± 3.86	4.41 ± 2.26*	13.14 ± 7.80*†	8.64 ± 5.08†	7.05 ± 4.35	7.17 ± 4.46†
	CX1942	8.23 ± 1.96	5.07 ± 1.31*	7.24 ± 2.92	8.60 ± 2.31†	8.23 ± 2.18†	8.37 ± 2.46†
PaO_2 mmHg (kPa)	Control	67 ± 3 (8.4 ± 0.4)	45 ± 7 (6.0 ± 0.9)*	51 ± 5 (6.8 ± 0.7)*	52 ± 4 (6.9 ± 0.5)*†	54 ± 5 (7.2 ± 0.7)*†	55 ± 5 (7.3 ± 0.7)*†
	Doxapram	69 ± 3 (9.2 ± 0.4)	46 ± 5 (6.1 ± 0.7)*	78 ± 8 (10.4 ± 1.1)*†	63 ± 9 (8.4 ± 1.2)†	61 ± 8 (8.1 ± 1.1)*†	63 ± 9 8.4 ± 1.2)†
	CX1942	67 ± 4 (8.4 ± 0.5)	46 ± 6 (6.1 ± 0.8)*	60 ± 7 (8.0 ± 0.9)†	65 ± 7 (8.7 ± 0.9)†	65 ± 9 (8.7 ± 1.2)†	63 ± 9 (8.4 ± 1.2)†
SaO2 (%)	Control	92 ± 1	75 ± 7*	80 ± 5*	82 ± 3*†	84 ± 4*†	85 ± 4*†
	Doxapram	92 ± 2	76 ± 4*	94 ± 3†	89 ± 4†	88 ± 5†	89 ± 6†
	CX1942	92 ± 3	78 ± 7*	88 ± 6†	90 ± 4†	90 ± 5†	90 ± 5†
$PaCO_2$ mmHg (kPa)	Control	33 ± 2 (4.4 ± 0.3)	44 ± 4 (5.9 ± 0.5)*	44 ± 4 (5.9 ± 0.5)*	45 ± 3 (6.0 ± 0.4)*	45 ± 2 (6.0 ± 0.3)*	44 ± 4 (5.9 ± 0.5)*
	Doxapram	33 ± 2 (4.4 ± 0.3)	43 ± 4 (5.7 ± 0.5)*	28 ± 5 (3.7 ± 0.7)*†	35 ± 6 (4.7 ± 0.8)†	35 ± 3 (4.7 ± 0.4)†	35 ± 5 (4.7 ± 0.7)†
	CX1942	32 ± 3 (4.4 ± 0.4)	44 ± 4 (5.9 ± 0.5)*	39 ± 5 (5.2 ± 0.7)*†	36 ± 3 (4.8 ± 0.4)†	35 ± 5 (4.7 ± 0.7)†	35 ± 4 (4.7 ± 0.5)†
pH	Control	7.49 ± 0.03	7.43 ± 0.03*	7.42 ± 0.04*	7.43 ± 0.03*	7.43 ± 0.02*	7.45 ± 0.03*
	Doxapram	7.50 ± 0.02	7.43 ± 0.02*	7.55 ± 0.05*†	7.48 ± 0.05†	7.50 ± 0.03†	7.50 ± 0.03†
	CX1942	7.50 ± 0.03	7.43 ± 0.03*	7.46 ± 0.05*	7.47 ± 0.03†	7.47 ± 0.04†	7.48 ± 0.03†

Table 2 The absolute values (mean ± standard deviation, $n = 8$) of respiratory rate (breaths minute^{-1}), tidal volume (L), ventilation (L minute^{-1}), PaO_2 (mmHg [kPa]), SaO_2 (%) $PaCO_2$ (mmHg [kPa]) and pH. The asterisk (*) indicates values that differ from those at -2 minutes and the † indicates the values that differ from those at 5 minutes for each trial.

DISCUSSION

Our study is one of the first to examine the effects of an ampakine on etorphine-induced respiratory depression. We found that the administration of CX1942 led to an improvement in respiratory parameters in etorphine-immobilized goats, but the improvement was not as rapid as that observed following the administration of doxapram. However, unlike doxapram, which led to some generalized arousal, CX1942 did not alter the level of immobilization in goats. CX1942 therefore appears to be a useful agent for improving respiration in opioid-immobilized animals.

Doxapram is widely used by veterinarians as a rescue agent when opioid-induced respiratory depression occurs. In our goats, intravenous doxapram led to a rapid improvement in all measured respiratory parameters, apart from tidal volume. We compared the novel agent CX1942, to doxapram. In response to the administration of CX1942, respiratory rate and tidal volume did not increase significantly, although presumably the summed interaction of these two variables resulted in the improvement in minute ventilation at 15, 20 and 25 minutes. By the end of the immobilization period, ventilation, PaO_2, SaO_2, $PaCO_2$ and pH in the goats that had received CX1942 were all better than before the administration of CX1942, and no different to values obtained before immobilization. Moreover, at 15, 20 and 25 minutes, there were no differences in any of the measured respiratory variables in the goats between the CX1942 and doxapram trials. However, arterial oxygen partial pressure following CX1942 administration were higher than those for the control trial at 20 and 25 minutes, while following doxapram administration the same parameters were no different to those of the control trial at these time points.

We have therefore demonstrated that CX1942 attenuates etorphine-induced respiratory depression in goats, and that it may be useful for improving the welfare of immobilized wild ungulates. Previous studies investigating the effects of etorphine on respiratory and cardiovascular systems have used goats as a model for wild ungulates, because use of a domesticated animal in a controlled setting limits the confounding effects of a capture-induced stress response, and allows for better determination of a drugs' pharmacodynamic effects (Heard et al. 1990; Heard et al. 1996; Buss & Meltzer 2001; Meyer et al. 2015). However, the degree of sensitivity to opioid-induced respiratory depression varies

amongst species (Burroughs et al. 2012). Thus, it is important that ampakines, such as CX1942, are ultimately tested in species such as white rhinoceros (*Ceratotherium simum*) and impala (*Aepyceros melampus*), which are particularly sensitive to the respiratory depressant effects of opioids (Meyer et al. 2010; Haw et al. 2014).

Although goats may not be as sensitive to opioids as impala, there are similarities with opioid pharmacodynamics in these two species. The initial etorphine-induced decrease in PaO_2 and SaO_2, with a minimum value at 5 minutes post administration, followed by a gradual increase in oxygenation as in our control trial, is similar to that observed in impala (Meyer et al. 2010). The improvement in oxygenation in goats during the control trial, however, was mild and the etorphine-induced hypoxaemic response was not corrected at any time point during the immobilization period. Therefore, supportive treatments are often deemed necessary in opioid-immobilized ungulates.

Doxapram, which is primarily a potassium channel antagonist (Van der Schier et al. 2014), is a respiratory stimulant commonly used by wildlife veterinarians in opioid-immobilized animals (Burroughs et al. 2012). It exerts its effects centrally, on the brainstem respiratory centres, and peripherally, on carotid and aortic chemoreceptors (Yost 2006). The profound, short-lived respiratory stimulatory effects as observed in our study were not unexpected. Early experiments in dogs revealed that doxapram is metabolized very quickly when administered intravenously (Bruce et al. 1965). In neonatal calves, doxapram was found to increase ventilation within 30 to 60 seconds of its administration (Bleul et al. 2010; Bleul & Byland 2012). Indeed, $PaCO_2$ in calves fell from about 47 mmHg to less than 30 mmHg in just 30 seconds, after which $PaCO_2$ started increasing again (Bleul et al. 2010).

Ampakines, such as CX1942, on the other hand, have a very different mode of action to that of doxapram. The ampakine family of compounds modulates AMPA receptors by binding to an allosteric site within the receptor complex, thereby increasing the duration of glutamate-induced AMPA receptor-gated inward currents (Arai et al. 2004). The respiratory stimulatory effects of ampakines are likely widespread, including their action on AMPA receptors within the pre-Bötzinger complex, where it is believed that the primary generation of respiratory rhythm occurs (Smith et al. 1991; Feldman et al. 2013).

That CX1942 did not significantly improve tidal volume or respiratory rate in our study is unexpected. Previous studies of other ampakines in rodent models have shown that ampakines act as a powerful stimulant of respiratory frequency and tidal volume after respiratory depression induced by mu-opioid receptor agonists (Ren et al. 2006; Ren et al. 2009). Individual variation in the sensitivity towards CX1942 may have accounted for the lack of observed differences in tidal volume and respiratory rate in our goats following CX1942 administration. Also, unlike doxapram, ampakines do not alter the normal frequency or amplitude of breathing in animals that do not have respiratory depression (Ren et al. 2006; Ren et al. 2015). We therefore suggest testing ampakines in species that experience greater respiratory depressant effects than do goats. Conversely, doxapram may stimulate respiration to above normal levels, as indicated by the hypocapnia and respiratory alkalosis, which occurred two minutes after doxapram administration in our study. Indeed, doxapram has been found to cause respiratory alkalosis in humans (Yost 2006), and hypocapnia ($PaCO_2 < 35$ mmHg) in neonatal calves (Bleul et al. 2010). A potential negative consequence of hypocapnia is cerebral vasoconstriction with subsequent cerebral hypoxia (Laffey & Kavanagh 2002). Based on the relatively short duration of effects of doxapram, it is unlikely that prolonged severe hypocapnia and cerebral hypoxia would occur, but it is important to be aware of this potential side-effect of doxapram, especially when working with animals that are physiologically compromised by age or illness.

Another adverse effect of doxapram is that it may lead to increased cardiac oxygen consumption (Bleul et al. 2010) as a result of the doxapram-induced increased cardiac workload associated with hypertension and increased cardiac output (Van der Schier et al. 2014), probably as a result of catecholamine release (Yost 2006). The use of doxapram for the improvement of oxygenation is therefore considered with caution nowadays in human medicine (Yost 2006). Apart from the cardiovascular alterations, doxapram is known to have a generalized central nervous system stimulatory effect, and, indeed, in human medicine, doxapram has been used to arouse patients in the post-anaesthetic setting as well as for emergency treatment of drug-induced central nervous system depression (Yost 2006). During wildlife immobilization, the potential arousal effects of doxapram are undesirable.

Based on our crude score of the depth of immobilization in the goats, doxapram caused more movement compared to CX1942. Another ampakine, CX717 was found to augment sedation in humans when it was used to prevent alfentanil-induced respiratory depression (Oertel et al. 2010). Although we were unable to measure the level of analgesia in our goats, evidence from both human (Oertel et al. 2010) and rat (Ren et al. 2009) studies suggests that ampakines do not reduce opioid analgesia.

Ultimately, an ideal pharmacological solution to opioid-induced respiratory depression in immobilized wildlife should be a drug potent enough to fit in a dart with the immobilizing cocktail, and it should not interfere with the opioid's ability to induce catatonic immobilization. In this way opioid-induced respiratory depression, which is most severe at the beginning of the immobilization period (Meyer et al. 2010), could be prevented, rather than treated, thereby significantly reducing the risk of capture-induced complications.
We administered the supportive treatments after etorphine had taken effect, so that we could determine if CX1942 could reverse opioid-induced respiratory depression in ungulates. These drugs are currently not in a formulation suitable for co-administration with the opioid in a dart. Moreover, if put in a dart, doxapram's duration of action is so short that its peak effects would have passed by the time the animal became completely immobile, and the general central nervous system stimulatory effects of doxapram may delay the onset of immobilization. CX1942, in comparison, has a slower onset of action, but it lacks solubility and therefore the volume required of this particular ampakine is too large for delivery in a dart.

Ampakines, such as CX1942, may indeed play an important role in counteracting opioid-induced respiratory depression in wildlife without causing unwanted arousal. If a water-soluble and potent formulation that can be combined in a dart mixture can be found, prevention and not only reversal of opioid-induced respiratory depression, as we have demonstrated with CX1942, could be achieved. By correcting etorphine's hypoxaemic effects without reversing immobilization, ampakines may offer a much-needed solution to prevent life-threatening opioid-induced respiratory depression that occurs during chemical immobilization of wildlife.

ACKNOWLEDGEMENTS

We thank the staff of the Central Animal Services of the University of the Witwatersrand for their animal management and technical support, and staff and students of the Brain Function Research Group for their help with animal handling and data collection. We also thank Dr Jun Ren, Research Associate at the University of Alberta, for his guidance and help in acquiring CX1942. Thanks also to Dr. Arnold Lippa of Cortex Pharmaceuticals for providing CX1942. This work was funded by a Faculty Research Committee Grant, University of the Witwatersrand, awarded to AH and a Thuthuka grant from the National Research Foundation, South Africa, awarded to LCRM. The authors declare that they have no competing interests.

REFERENCES

Arai AC, Xia YF & Suzuki E (2004) Modulation of AMPA receptor kinetics differentially influences synaptic plasticity in the hippocampus. *Neuroscience* 123, 1011-1024.

Bleul U, Bircher B, Jud RS & Kutter APN (2010) Respiratory and cardiovascular effects of doxapram and theophylline for the treatment of asphyxia in neonatal calves. *Theriogenology* 73, 612-619.

Bleul U & Bylang T (2012) Effects of doxapram, prethcamide and lobeline on spirometric, blood gas and acid-base variables in healthy new-born calves. *Vet J*. 194, 240-246.

Bruce RB, Pitts JE, Pinchbeck F & Newman J (1965) Excretion, distribution, and metabolism of doxapram hydrochloride. *J Med Chem.* 8, 157-164.

Burroughs R, Meltzer D & Morkel P (2012) Applied pharmacology. In *Chemical and Physical Restraint of Wild Animals* (2nd edn). Kock MD, Meltzer D & Burroughs R (eds). South Africa: IWVS (Africa), pp 53-62.

Buss P & Meltzer D (2001) Changes in respiratory function following intramuscular administration of etorphine in boer goats (*Capra hircus*). *J S Afr Vet Assoc*. 72, 137-142.

Dahan A, Aarts L & Smith TW (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 112, 226-38.

El Mallah MK, Pagliardini S, Turner SM, Cerreta AJ, Falk DJ, Byrne BJ, Greer JJ & Fuller DD (2015) Ampakines enhance respiratory output in a murine model of Pompe disease. *Am J Respir Cell Mol Biol*. In press

Feldman JL, Del Negro CA & Gray PA (2013) Understanding the rhythm of breathing: so near, yet so far. *Annu Rev Physiol*. 75, 423-52.

Funk GD, Smith JC & Feldman JL (1993) Generation and transmission of respiratory oscillations in medullary slices: role of excitatory amino acids. *J Neurophysiol*. 70, 1497-1515.

Greer JJ, Smith JC & Feldman JL (1991) Role of excitatory amino acids in the generation and transmission of respiratory drive in neonatal rats. *J Physiol*. 437, 727-749.

Haw A, Hofmeyr M, Fuller A, Buss P, Miller M, Fleming G & Meyer L (2014) Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*). *BMC Vet Res.* 10, 253.

Heard DJ, Kollias GV, Buss D, Caligiui R & Coniglario J (1990) Comparative cardiovascular effects of intravenous etorphine and carfentanil in domestic goats. *J Zoo Wildl Med*. 21, 166-170.

Heard DJ, Nichols WW, Buss D & Kollias GV (1996) Comparative cardiopulmonary effects of intramuscularly administered etorphine and carfentanil in goats. *Am J Vet Res*. 57, 87-96.

Kilkenny C, Browne WJ, Cuthill IC, Emerson M. & Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412.

Laffey JG & Kavanagh BP (2002) Hypocapnia. *N Engl J Med*. 347, 43-53.

Lynch G (2006) Glutamate-based therapeutic approaches: ampakines. *Curr. Opin. Pharmacol*. 6, 82-88.

Meyer LCR, Fuller D & Mitchell D (2006) Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats. *Am J Physiol Regul Integr Comp Physiol*. 290, R405-R413.

Meyer LCR, Hetem RS, Fick LG, Mitchell D & Fuller A (2010) Effects of serotonin agonists and doxapram on respiratory depression and hypoxemia in etorphine-immobilized impala (*Aepyceros melampus*). *J Wildl Dis*. 46, 514-524.

Meyer LCR, Hetem RS, Mitchell D & Fuller A (2015) Hypoxia following etorphine administration in goats (*Capra hircus*) results more from pulmonary hypertension than from hypoventilation. *BMC Vet Res*. 11, 18.

Oertel BG, Felden L, Tran PV, Bradshaw MH, Angst MS, Schmidt H, Johnson S, Greer JJ, Geisslinger G, Varney MA & Lötsche J (2010). Selective antagonism of opioid-induced ventilatory depression by an ampakine molecule in humans without loss of opioid analgesia. *Clin Pharmacol Ther*. 87, 204-211.

Pace RW, Mackay DD, Feldman JL, & Del Negro CA (2007) Inspiratory bursts in the preBötzinger complex depend on a calcium-activated non-specific cation current linked to glutamate receptors in neonatal mice. *J Physiol.* 582, 113-25.

Pattinson KTS (2008) Opioids and the control of respiration. *Br J Anaesth*. 100, 747-758.

Ren J, Poon BY, Tang Y, Funk GD & Greer JJ (2006) Ampakines alleviate respiratory depression in rats. *Am J Respir Crit Care Med*. 174, 1384-91.

Ren J, Ding X, Funk GD & Greer JJ (2009) Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. *Anesthesiology* 110, 1364-70.

Ren J, Ding X & Greer JJ (2012) Respiratory depression in rats induced by alcohol and barbiturate and alleviation by ampakine CX717. *J Appl Physiol.* 113, 1004-11.

Ren J, Lenal F, Yang M, Ding X & Greer JJ (2013) Co-administration of the ampakine CX717 with propofol reduces respiratory depression and fatal apneas. *Anesthesiology* 118, 1437-45

Ren J, Ding X & Greer JJ (2015) Ampakines enhance weak endogenous respiratory drive and alleviate apnea in perinatal rats. *Am J Respir Crit Care Med.* 191, 704-710.

Smith JC, Ellenberger HH, Ballanyi K, Tichter DW & Feldman JL (1991) Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* 254, 726-729.

Van der Schier R, Roozekrans M, Van Velzen M, Dahan A & Niesters M (2014) Opioid-induced respiratory depression: reversal by non-opioid drugs. *F1000 Prime Rep*. 6, 79.

Yost CS (2006) A new look at the respiratory stimulant doxapram. *CNS Drug Rev.* 12, 236-249.

CHAPTER 6

Discussion

6.1 The opioid dilemma: nature of the problem

Opioid drugs play a significant role in both human and veterinary medicine. Their unparalleled analgesic effects are fundamental to the treatment of severe and chronic pain syndromes, particularly in hospital settings. In veterinary medicine, the advent of highly potent synthetic and semi-synthetic opioid drugs in the 1960s markedly facilitated the advancement of wildlife capture techniques. For the first time, a small volume of drug could induce a rapid, reversible catatonic immobilization in large herbivores, which significantly decreased animal mortalities during capture.

Potent opioids, such as etorphine and thiafentanil, have proved especially beneficial for the implementation of many conservation strategies, particularly for mega-herbivores such as rhinoceros and elephant. Today, as rhinoceros poaching is escalating at an exponential rate (Fig. 6.1), potent opioids remain integral to anti-poaching strategies such as translocations, identity marking, dehorning, veterinary treatments and the placement of tracking devices.

However, the wildlife industry extends far beyond the conservation of individual species. In the 1960s, approximately the same time as etorphine was introduced, legislative changes in southern African countries resulted in a massive shift away from livestock farming towards game ranching (Lindsey et al. 2009). The growth in private game farms in southern Africa has resulted in significant increases in the abundance and distribution of many wildlife species. Game ranching is a diverse sector that combines ecotourism, the sale of live animals, several forms of hunting, and meat production (Child et al. 2012). Wildlife is a more profitable and environmentally sustainable form of land use than is livestock farming, especially in semi-arid savannas (Child et al. 2012).

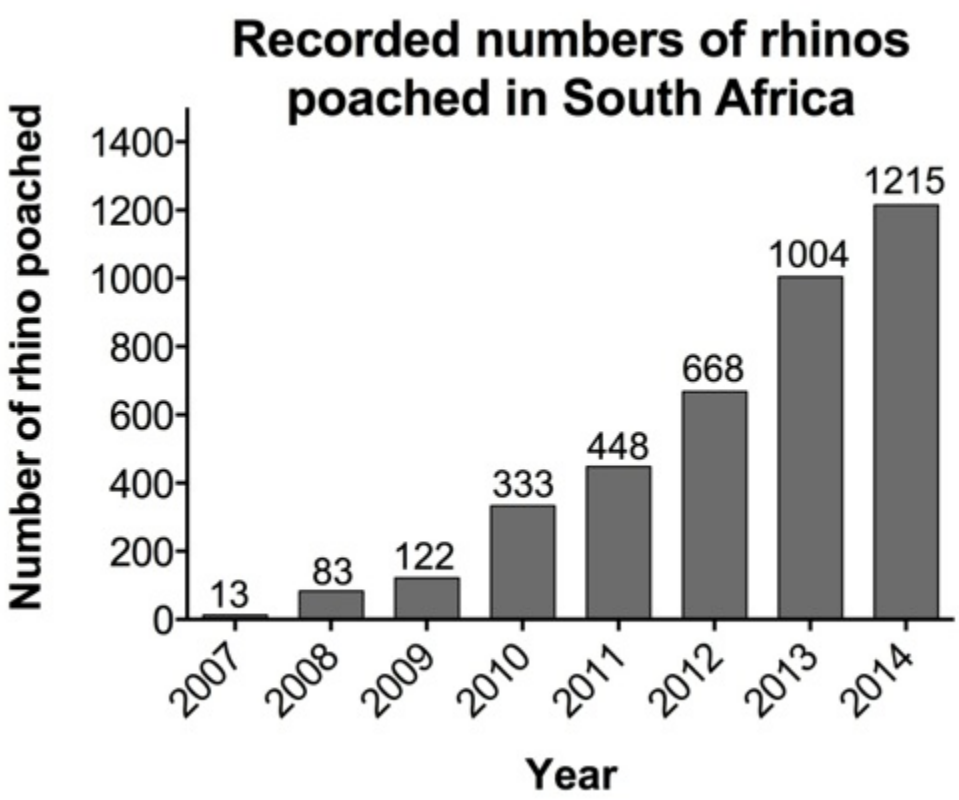


Figure 6.1: The number of rhinoceros poached in South Africa from 2007 to 2014. Data from the South African Department of Environmental Affairs. <https://www.environment.gov.za> [20 December 2015].

According to global climate change predictions, droughts will become more prevalent and by 2080 there will be an increase of between 5 % and 8 % of arid and semi-arid land in Africa (IPCC 2007). Currently, cattle make up the majority of livestock biomass in Africa, but cattle are poorly adapted to drought conditions (Kay 1997). As grazers, cattle do not feed on trees and shrubs that provide the best forage in a drought, and they are heavily reliant on drinking water (Kay 1997). Conversely, many wild ungulates are able to forage on a diversity of trees, shrubs and grasses, and are much less reliant on drinking water than are cattle. Thus, as droughts become more frequent in southern Africa, it is highly probable that livestock farms will increasingly be converted to more ecologically sustainable and economically viable game ranches. Furthermore, the wildlife industry creates more jobs than does the traditional livestock industry; an added benefit in the face of rising unemployment (Child et al. 2012).

Currently, about 10,000 private wildlife ranches cover an estimated 16.8 % of agricultural land (about 20.5 million hectares) in South Africa, and the African game ranching sector has expanded between 5 % and 20 % annually in the last decade (Child et al. 2012, Dry 2015). In order to stock and manage these wildlife ranches, animals need to be safely immobilized for translocation and veterinary interventions. Buying and selling game species is a lucrative business in South Africa. In 2014, the total turnover on game auctions grew by 35 % year on year to a historic high of R1.8 billion (Janovsky 2015) (Fig 6.2). The majority of the species sold at these auctions is large herbivores, which can be sold and transported only after immobilization using potent opioids, such as etorphine and thiafentanil (Table 6.1). Thus, not only are opioids essential for the implementation of critical conservation strategies, they also are central to the growth of a rapidly expanding, economically important private wildlife industry.

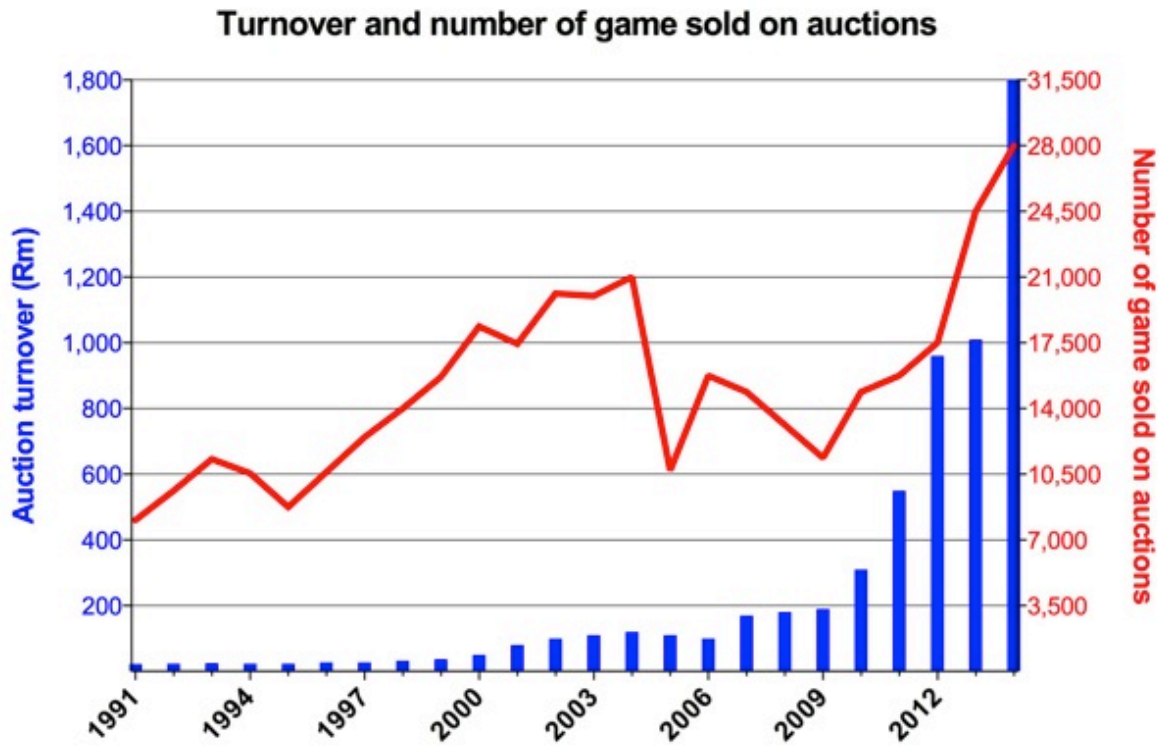


Figure 6.2. Growth of the wildlife industry, showing the monetary turnover (blue) and number of game animals sold (red) at game auctions in South Africa from 1991 to 2014.

Figure redrawn from Janovsky E (2015) "Wildlife industry expected to continue to grow" (ABSA) < http://www.absa.co.za/deployedfiles/Absacoza/PDFs/Commercial/Sector.pdf> [25 January 2016].

Game species	Most common immobilizing agent	Sensitive to OIRD*
Blesbok (*Damaliscus pygargus phillipsi*)	Etorphine	✔
Blue wildebeest (*Connochaetes taurinus*)	Etorphine	✔
Buffalo (*Syncerus caffer*)	Etorphine	✔✔
Eland (*Taurotragus oryx*)	Thiafentanil	✔
Gemsbok (*Oryx gazella*)	Etorphine/Thiafentanil	✔
Giraffe (*Giraffa camelopardalis*)	Etorphine/Thiafentanil	✔✔
Impala (*Aepyceros melampus*)	Etorphine/Thiafentanil	✔✔
Kudu (*Tragelaphus strepsiceros*)	Etorphine/Thiafentanil	✔
Nyala (*Tragelaphus angasii*)	Thiafentanil	✔
Red hartebeest (*Alcelaphus buselaphus caama*)	Etorphine/Thiafentanil	✔
Roan (*Hippotragus equinus*)	Etorphine/Thiafentanil	✔
Sable (*Hippotragus niger*)	Etorphine/Thiafentanil	✔
Springbok (*Antidorcas marsupialis*)	Etorphine	✔✔
Waterbuck (*Kobus ellipsiprymnus*)	Thiafentanil	✔
White rhinoceros (*Ceratotherium simum*)	Etorphine	✔✔
Zebra (*Equus quagga*)	Etorphine	✔

Table 6.1. **The species most commonly traded at game auctions in South Africa in 2015.** All these species are sensitive to opioid-induced respiratory depression (*OIRD) (✔) with some species displaying greater sensitivity (✔✔) than others. Data derived from Burroughs et al. 2012a and "2015 average game sale prices" <www.vleissentraal.co.za> [23 January 2016].

Unfortunately, the beneficial effects of opioids in both human and veterinary medicine are coupled with potentially life-threatening side-effects; most notably, respiratory depression (Bowdle 1998; Burroughs et al. 2012b; Dahan et al. 2010; Van der Schrier et al. 2014). Etorphine and thiafentanil appear to cause similar cardiorespiratory effects in wildlife species (Meyer et al. 2008), although few comparative studies have been done. Furthermore, some of the most valuable species, such as buffalo, impala, giraffe and white rhinoceros, are known to be particularly sensitive to opioid-induced respiratory depression (Burroughs et al. 2012a). In healthy white rhinoceros, we have recorded arterial partial pressures of oxygen as low as 25 mmHg (chapter 3). Although these rhinoceros survived, they were clearly physiologically compromised.

This opioid-induced respiratory depression constrains the ability for opioids to be used to their full beneficial potential (Pasternak 2014). In the United States of America human deaths from prescription opioids have been rising sharply, from about 4,400 (12 individuals per day) in 1999 to 16,000 (44 individuals per day) in 2010 (Volkow et al. 2014). This rise in the number of opioid-related deaths has paralleled the increasing pressure for clinicians to become more proactive in identifying and treating chronic pain (Okie 2010). The main cause of opioid fatalities is cardiorespiratory arrest initiated by opioid-induced respiratory depression leading to hypoxia and hypercapnia (Dahan et al. 2010; Van der Schrier et al. 2014). Although the number of opioid-related fatalities is well recorded, the number of near-fatal events remains unknown, but non-fatal opioid-induced respiratory events are estimated to exceed fatal events by at least a factor of 100 (Van der Schrier et al. 2014). Similarly, in veterinary medicine, opioid-induced respiratory depression causes morbidity and mortality, with worsening hypoxaemia noted as a risk factor for mortality (Burroughs et al. 2012a; Kock et al. 1995). The opioid dilemma, therefore, is that potent opioids such as morphine, fentanyl, etorphine and thiafentanil are the only agents capable of inducing profound analgesia, sedation and rapid, reversible immobilization; but they also induce life-threatening respiratory depression.

In wildlife, the need to immobilize healthy animals will continue to increase, as described above, but so too will the need to immobilize physiologically-compromised animals. As rhinoceros poaching escalates, more critically injured animals will require opioid-induced immobilization for treatment and translocation. Severe stress, such as haemorrhagic shock, trauma and bacterial infection, may result in a massive release of endogenous opioids

(Feng et al. 2013). Darting such an animal with exogenous opioids will place the animal at an increased risk of opioid-induced mortality caused by cardiorespiratory arrest. Changing climatic conditions and irregular rainfall, as is predicted to occur in much of Africa (IPCC 2007), also may result in more diseased and nutritionally-compromised wild animals. In an effort to save species from succumbing to the ills of a rapidly changing climate, these physiologically stressed animals may require immobilization. The compounding effects of opioid-induced respiratory depression on physiologically compromised animals may lead to an increased risk of capture-induced morbidity and mortality.

In order to harness the full beneficial effects of potent opioids, both human and veterinary clinicians need the freedom to use opioids without the fear of inducing life-threatening respiratory compromise. To achieve this ideal, rigorous, species-specific research that investigates the use of opioids in patients under different physiological conditions, is required, and indeed essential for improved animal, human and environmental health.

6.2 Recommendations for solving the opioid dilemma

Opioid-induced respiratory depression primarily refers to the central opioid-induced neuronal depression that leads to reduced respiratory frequency with consequent hypercapnia. However, opioid drugs not only reduce the rate of breathing, but also obtund the respiratory responsiveness to carbon dioxide and hypoxaemia, reduce pulmonary compliance, increase upper airway resistance and reduce the diffusion capabilities of oxygen from the alveoli to the arterial blood, thus causing hypoxaemia and hypercapnia that compromise normal cellular respiration (Dahan et al. 2010; Feng et al. 2013; Meyer et al. 2015). Therefore “opioid-induced respiratory compromise” or “opioid-induced respiratory impairment” are more appropriate terms to use than is “opioid-induced respiratory depression”, when discussing the overall effects opioids have on normal respiration (MacIntyre et al. 2011). As described above, this generalised opioid-induced respiratory compromise can be life-threatening, and is the opioid side-effect most feared by both human and animal clinicians. Prevention or treatment of opioid-induced respiratory compromise is therefore of critical importance.

Full-opioid antagonists, such as naltrexone and naloxone, will reverse opioid-induced respiratory compromise, but they will also reverse the desirable effects of full opioid

agonists such as analgesia, sedation and catatonia. Mixed opioid agonist-antagonists that maintain the full agonist's positive effects, while reversing the negative effects, such as respiratory depression, appear to be one approach to treating opioid-induced respiratory compromise.

Of the available opioid agonist-antagonists, butorphanol is currently the most accessible and widely used in the treatment of opioid-induced respiratory compromise in immobilized herbivores (Burroughs et al. 2012b). However, another opioid agonist-antagonist, nalbuphine, which is not currently used in veterinary medicine, has been advocated as a better mu-opioid antagonist than is butorphanol (Bowdle et al. 1987; Pallasch & Gill 1985; Schmidt et al. 1985; Zucker et al. 1987). However, my findings did not support that idea. I found that in etorphine-immobilized goats, butorphanol, at a lower dose than nalbuphine, corrected the arterial partial pressure of oxygen (P_aO_2), hypopnea and hypoventilation induced by etorphine, whereas nalbuphine corrected only P_aO_2. Thus, in our ungulate model, we advocate the use of butorphanol as the preferred opioid agonist-antagonist for reversing etorphine-induced respiratory compromise. For a more comprehensive study, it would have been ideal to assess the effects of nalbuphine and butorphanol with oxygen insufflation, as carried out in the rhinoceros study. However, our goal in this specific goat study was to compare the respiratory effects of butorphanol with nalbuphine, and therefore we did not want the confounding effects of oxygen insufflation. It is also possible that the efficacy of opioid agonist-antagonists in reversing opioid-induced respiratory compromise may depend on the species. Indeed, results from studies in mice (Schmidt et al. 1985) and humans (Bowdle et al. 1987) indicated that nalbuphine may be a more potent mu-antagonist than is butorphanol. Also, in humans anaesthetised with nitrous oxide, butorphanol caused significantly greater respiratory depression than did nalbuphine when administered at equianalgesic doses (Zucker et al. 1987). These conflicting results between species reveal the complexity of opioid pharmacology, especially with regards to opioid agonist-antagonists that may vary in their affinity and efficacy for different opioid receptors under different physiological conditions, both within and between species.

A drawback of the use of opioid agonist-antagonists in immobilized animals is that the reversal of respiratory compromise may be accompanied by increased arousal, as found in goats in our study (chapter 2). The increased oxygen consumption and carbon dioxide

production in the goats immediately after the administration of butorphanol and nalbuphine indicated that metabolism increased as the level of immobilization decreased. My findings therefore support the view that currently the desirable effects of mu-opioid receptor activation, such as analgesia and catatonia, cannot be completely separated from the adverse effects, such as respiratory depression (Kieffer 1999).

Although opioid agonist-antagonists do not appear to be the "silver bullet" for treating opioid-induced respiratory compromise, wildlife veterinarians have found butorphanol to be beneficial in improving some cardiorespiratory parameters, such as respiratory frequency, especially in the most opioid-sensitive species, such as white rhinoceros. The increasing need to immobilize rhinoceros in the face of rising poaching pressure has necessitated the need to find the best available immobilization protocol specific to white rhinoceros. Before I conducted my studies, some research aimed at verifying anecdotal findings for white rhinoceros immobilization had already been conducted. Specifically, the effects of butorphanol (Miller et al. 2013; Wenger et al. 2007) and oxygen insufflation (Bush et al. 2004) had been assessed during field immobilizations. Although field trials are important, and yield results that add critical contributions to our knowledge of wildlife immobilization, it is difficult to make accurate comparisons between different interventions in field trials. Variability such as terrain, dart placement, physical exertion by the animal, animal position and individual variability during different field immobilizations will undoubtedly influence the animal's physiology. Controlled, prospective, cross-over trials are essential to evaluate and compare different treatment interventions. However, such scientifically rigorous trials are seldom carried out because of the difficulty in establishing a controlled environment and the expense and risk of immobilizing animals outside of routine management procedures. I therefore evaluated the use of butorphanol with and without oxygen insufflation in a cross-over trial in boma-kept immobilized white rhinoceros of similar age and sex (chapter 3).

An important factor that I considered, when assessing the cardiorespiratory status of the recumbent rhinoceros, was animal position. In anaesthetised horses, sternal recumbency results in improved alveolar ventilation, oxygenation and systemic perfusion, compared to lateral recumbency (Gleed & Dobson 1988). Similarly, the hypoxaemia observed in immobilized rhinoceros is less severe in animals kept in a sternal position, compared to those in a lateral posture (Morkel et al. 2010; Wenger et al. 2007). Laterally recumbent

rhinoceros are thought to have greater alveolar dead space as a result of decreased lung perfusion (Morkel et al. 2010). We chose to conduct our trials in laterally recumbent rhinoceros, as we wanted to determine if a treatment could correct the most severe forms of etorphine-induced respiratory compromise. Also, sternal recumbency results in decreased blood flow to the limbs, which may lead to additional complications, such as myopathy (Morkel et al. 2010). Although recumbency will have an effect on the blood oxygenation of large animals, we found, like others (Miller et al. 2013), that immobilized rhinoceros in the standing position were still severely hypoxaemic (P_aO_2 = 58.4 ± 9.1 mmHg).

We also found that butorphanol administration at the start of the immobilization period attenuated, but did not correct, the respiratory compromise that usually occurs in chemically-immobilized white rhinoceros. Twenty minutes into the immobilization period, the rhinoceros were still critically hypoxaemic (P_aO_2 = 64 ± 9 mmHg). Similarly, butorphanol reduced the severity of the etorphine-induced hypercapnia in immobilized rhinoceros, but the rhinoceros were still hypercapnic (P_aCO_2 = 68 ± 3 mmHg) at 20 minutes. Butorphanol alone, therefore, is not adequate for the complete reversal of opioid-induced respiratory compromise, as indicated by other studies that were conducted in the field (Miller et al. 2013; Wenger et al. 2007).

Oxygen insufflation has also been advocated as an important treatment for opioid-induced respiratory compromise during chemical immobilization of wildlife (Bush et al. 2004; Fahlman 2008). Surprisingly, we found that oxygen as a sole support, administered directly into the trachea at a flow rate of 30 L/min, had a detrimental effect on the rhinoceros' respiratory physiology. After oxygen insufflation was initiated, the respiratory rate in the rhinoceros decreased to about 3 breaths/min and the hypercapnia was exacerbated with peak arterial carbon dioxide partial pressures of 123 ± 12 mmHg. Moreover, P_aO_2 values during oxygen insufflation were not different from those recorded in the rhinoceros when no supportive treatment was administered. We suggest that absorption atelectasis underlies these unexpected results.

Absorption atelectasis occurs when the gases in the alveoli are absorbed faster than they can be replaced. Air is made up of a mixture of gases, the majority of which is nitrogen, and these gases are not absorbed into the blood at an equal rate. Oxygen is absorbed more

quickly than is nitrogen so the rate of absorption of gas from the alveoli into the blood increases with increasing fraction of inspired oxygen (FiO_2) (Nyman et al. 1990). As nitrogen is not readily absorbed into the blood, the presence of nitrogen acts as a stent to keep the alveoli open. As the concentration of oxygen in the alveoli increases (with increased FiO_2), and nitrogen decreases, so too will nitrogen's stenting effect decrease. Also, when the arterial partial pressure of oxygen is very low (as in etorphine-immobilized animals) the pressure gradient of oxygen from the alveoli to the pulmonary arterial blood will be very high, thus further increasing the amount and speed at which oxygen is absorbed from the alveoli (Wilson & Benumof 2005). If the alveoli gases are absorbed faster than they can be replaced, atelectasis will occur. In horses under general anaesthesia where airway integrity was compromised by the weight of abdominal organs, increased intrapulmonary shunting occurred as a result of atelectasis when breathing high FiO_2 (>0.8) compared to a lower FiO_2 (0.21-0.3) (Marntell et al. 2005; Suter et al. 1975;). In addition, lower minute ventilation in horses receiving supplemental oxygen resulted in higher FiO_2 (Staffieri et al. 2009). We therefore propose that the rhinoceros receiving oxygen supplementation in our study had a high FiO_2 together with narrowed or compressed airways as a result of pressure from abdominal organs and poor ventilation. The gases in the alveoli of our immobilized rhinoceros were likely absorbed faster than they could be replaced and atelectasis ensued. We suggest that the rhinoceros receiving oxygen as the only intervention had significant intra-pulmonary shunt fractions, probably in the region of 50% or greater (Fahlman 2008).

Another consequence of a high shunt fraction is hypercapnia. Gaseous exchange (both oxygen absorption and carbon dioxide removal) cannot occur if the pulmonary blood perfuses non-ventilated alveoli. The hypercapnia in our rhinoceros that received oxygen insufflation as a sole support was severe, with P_aCO_2 peaking at 123 ± 12 mmHg (normal P_aCO_2 range = 44 – 54 mmHg). Hypercapnia can have significant, negative physiological consequences, including the release of catecholamines and leakage of potassium from cells into plasma, which decreases myocardial contractility and predisposes the animal to arrhythmias and ultimately heart failure (Johnson & De Morais 2012; Wilson & Benumof 2005). Under normal circumstances, an increasing partial pressure of carbon dioxide in the blood should stimulate ventilation. However, full opioid agonists, such as etorphine, significantly reduce chemoreceptor sensitivity and therefore normal ventilatory control is inhibited during opioid-induced immobilization (Kukanich & Papich 2009). This

inhibition of ventilatory control, however, does not explain why the respiratory rate in rhinoceros that received oxygen decreased from about 8 breaths per minute before oxygen administration to about 3 breaths per minute at 15 minutes when P_aCO_2 was at its highest and P_aO_2 was only 44 ± 12 mmHg. Reasons for this further decrease in respiratory rate are not clear, but we hypothesize that it was directly related to the extreme hypercapnia, which caused a central depression of respiration (Merriman 1955; Wilson & Benumof 2005).

Although oxygen insufflation on its own could not be used to treat respiratory compromise, we demonstrated that the immobilization-induced hypoxaemia in white rhinoceros could be completely corrected by administering intravenous butorphanol shortly after recumbency and supplying oxygen throughout the immobilization period via nasotracheal intubation. The synergistic effect of oxygen and butorphanol far surpassed the benefit of either agent used individually and, for the first time, we consistently abolished the etorphine-induced hypoxaemia in boma-immobilized white rhinoceros. This marked increase in the partial pressure of arterial oxygen most likely resulted from the improved tidal volume and chest expansion induced by the butorphanol, which facilitated the high concentration of oxygen in the alveoli to dissolve into the blood without causing excessive atelectasis. Therefore, in the confined boma setting (Fig. 6.3), where rhinoceros can be darted with ease and without excessive animal exertion, I recommend intravenous butorphanol combined with oxygen insufflation as the best available treatment to reverse hypoxaemia after etorphine-induced immobilization.



Fig 6.3: Sub-adult male white rhinoceros immobilized in a boma
(Photo credit: Andrea Fuller)

In southern Africa, however, the majority of rhinoceros are immobilized in free-ranging conditions, thus it was imperative that we tested our treatment in free-ranging rhinoceros to determine if butorphanol combined with oxygen offered advantages over other field protocols. Similar to boma-immobilized rhinoceros, we demonstrated that the hypoxaemia that occurs in chemically immobilized free-ranging white rhinoceros was significantly improved with the administration of intravenous butorphanol at the beginning of the immobilization together with oxygen insufflation throughout the recumbent period (chapter 4). However, in field-immobilized rhinoceros, our treatment did not fully correct the capture-induced hypoxaemia (P_aO_2 = 81 ± 24 mmHg, normal P_aO_2 range = 90 – 109 mmHg), but the peak arterial oxygen concentration recorded in our rhinoceros was higher than that found in other studies where partial reversal of etorphine and change in body position had been used to improve oxygenation (Fahlman 2008; Miller et al. 2013; Wenger et al. 2007). Also, rhinoceros may be better able to maintain adequate tissue oxygenation with lower P_aO_2 values than can smaller mammals as a result of their higher oxygen affinity for haemoglobin (lower P_{50}) and lower tissue metabolic rate (Ball et al. 2011; Baumann et al. 1984; Heard et al. 1992). Therefore, the improvement in arterial

oxygenation in the rhinoceros in our study may have been sufficient to prevent hypoxic damage to muscles and other vital organs, thereby reducing the risk of capture-related morbidity such as myopathy, renal failure, abortion and cardiac failure (Heard et al. 1992).

Unfortunately, it was not ethically possible to have a control group in the field, which needs to be considered in the interpretation of our results, given the disparity in effects of the identical treatments carried out in boma and field conditions. However, I believe the conclusions drawn from our field trial are valid, as the treatment intervention significantly improved the respiratory parameters compared to pre-intervention values, and the initial P_aO_2 was similar in both boma- and field-immobilized rhinoceros.

The disparity between the effects of the identical treatment intervention in field- and boma-immobilized rhinoceros may partly be explained by an increased stress response in field-immobilized rhinoceros. Activation of the sympathetic nervous system will induce pulmonary hypertension through vasoconstriction, via activation of alpha-1 and alpha-2 adrenoreceptors in the pulmonary vasculature (Hyman & Kadowitz 1986; Kadowitz & Hyman 1973; Salvi 1999). Increased sympathetic tone will also increase cardiac output resulting in greater blood flow to the lungs, thus further increasing pulmonary pressure in the vasoconstricted vessels. We hypothesize that in the field-immobilized rhinoceros, the psychological and physiological stress response associated with fleeing the helicopter resulted in a higher sympathetic outflow and pulmonary vasoconstriction compared to that in the habituated boma-immobilized rhinoceros, resulting in more severe pulmonary hypertension. Pulmonary hypertension, leading to pulmonary congestion and possibly oedema (Gao & Raj 2005), will reduce oxygen diffusion from the alveoli into the arterial blood (Meyer et al. 2015). Increased cardiac output resulting from sympathetic activation and exertion will also increase the speed of blood flow and result in decreased time for red blood cells to traverse alveoli capillaries in the pulmonary bed. If this transit time is less than 0.25s, oxygen will not have enough time to diffuse and equilibrate in the blood (West 2012). Thus, functional changes in the pulmonary vasculature of field-immobilized white rhinoceros may partly account for the differences observed in arterial oxygen concentration between field- and boma-immobilized rhinoceros after they received identical supportive treatments. In addition, exertion may result in hypermetabolism leading to extremely low partial pressures of oxygen in venous blood, which will

negatively affect P_aO_2, especially in the presence of pulmonary hypertension and increased shunt fractions.

Apart from oxygenation, the acidaemia in the field-immobilized rhinoceros was also initially worse than in the boma-immobilized rhinoceros. That acidaemia was worse in field-immobilized rhinoceros, despite a better ventilatory response compared to boma-immobilized rhinoceros (P_aCO_2 = 63 ± 8 mmHg in field-immobilized rhinoceros *versus* 79 ± 7 mmHg in boma-immobilized rhinoceros), suggests that metabolic acidosis is a significant contributor to the lower pH in field-immobilized white rhinoceros. Indeed, the significantly lower bicarbonate values in the field, compared to boma-immobilized rhinoceros also suggests that metabolic acidosis contributed to acid-base imbalances in field-immobilized rhinoceros. Capture teams should therefore endeavour to limit the intensity and duration of the helicopter chase during capture. Ideally, we should have had a control group in the field to determine how pH changed in the immobilized rhinoceros without any treatment intervention. I suspect pH may have improved slightly in control animals, as some of the lactate would be broken down over time. However, without sufficient oxygenation, as we suspect would occur in control animals, generalized hypoxia would have occurred, resulting in anaerobic metabolism leading to sustained, if not increased, levels of lactate in the immobilized rhinoceros.

Another suggestion from wildlife veterinarians working with animals in the field, is that diprenorphine could be used in place of butorphanol for the partial reversal of etorphine in immobilized white rhinoceros. Diprenorphine, which acts as an opioid agonist-antagonist in rhinoceros, is thought to have greater antagonistic effects than butorphanol. Moreover, diprenorphine (M5050®) is supplied with etorphine (M99®) by its manufacturer, Elanco (previously Novartis), and is registered as the standard antidote for etorphine-induced immobilization. To reverse the effects of etorphine, it is recommended that diprenorphine be given at 2-3 mg per mg etorphine (Burroughs et al. 2012a; Swan 1993). In most species, diprenorphine is the antagonist most commonly used for the reversal of immobilization (Burroughs et al. 2012b) and recovery times, following intravenous diprenorphine administration, are from a few seconds to four minutes (Swan 1993). I attempted to systematically compare the respiratory stimulatory effects of diprenorphine to those of butorphanol and nalbuphine in immobilized goats, but found that the smallest possible dose (0.01 mg/kg) of diprenorphine that could be practically administered

completely reversed etorphine's immobilization effects. However, as diprenorphine does appear to have some partial kappa-agonist activity (Lewis & Husbands 2004) in some species, a degree of sedation may be maintained in certain species. It is, therefore, recommended that the full opioid antagonist, naltrexone, be used to reverse opioid-induced immobilization in prey species that are released into an area with predators. In white rhinoceros diprenorphine does not antagonize the effects of etorphine completely and animals will remain partially narcotized for up to eight hours following the administration of diprenorphine (Kock et al. 1995; Rogers 1993).

Therefore, like butorphanol, diprenorphine has been advocated as a potential treatment to reverse the respiratory depressant effects of etorphine in immobilized white rhinoceros. Based on anecdotal findings from the field, practitioners suggest giving 1 mg diprenorphine with 10 mg butorphanol intravenously immediately to all immobilized white rhinoceros (Burroughs et al. 2012a). In addition to the studies described in chapters 2 to 5, we conducted some preliminary studies to determine if diprenorphine was a useful treatment for etorphine-induced respiratory compromise in immobilized white rhinoceros. To standardize our trial, we chose to give diprenorphine and butorphanol at a set ratio to the dose of etorphine used in the immobilizing dart. Therefore we used 0.4 mg diprenorphine per mg etorphine with 3.3 mg butorphanol per mg etorphine, which roughly equates to 1 mg diprenorphine with 10 mg butorphanol if 2-3 mg etorphine is used, as it was in our study.

We found that butorphanol + diprenorphine + oxygen is just as effective as butorphanol + oxygen in improving the respiratory physiology of etorphine-immobilized white rhinoceros (Fig. 6.4). Throughout the immobilization period, there were no differences in the P_aO_2 and the P_aCO_2 values in the rhinoceros that received butorphanol + diprenorphine + oxygen and those that received butorphanol+oxygen. Also, both these treatments led to a significant increase in P_aO_2, and decrease in P_aCO_2 compared to pre-intervention values. A practical advantage of using a much lower dose of butorphanol (3.3 x etorphine mg compared to 15 x etorphine mg) combined with diprenorphine is a significant cost reduction. As diprenorphine is supplied with etorphine, many wildlife veterinarians have unused bottles of diprenorphine in their possession as veterinarians often choose to reverse the effects of etorphine with the full-opioid antagonist, naltrexone, when releasing animals into areas with predators. Butorphanol, on the other hand, is an additional cost. Therefore,

although there is no physiological advantage, field veterinarians can be assured that using the cheaper supportive treatment intervention of butorphanol + diprenorphine + oxygen will afford as much benefit as administering butorphanol+oxygen to etorphine-immobilized white rhinoceros.

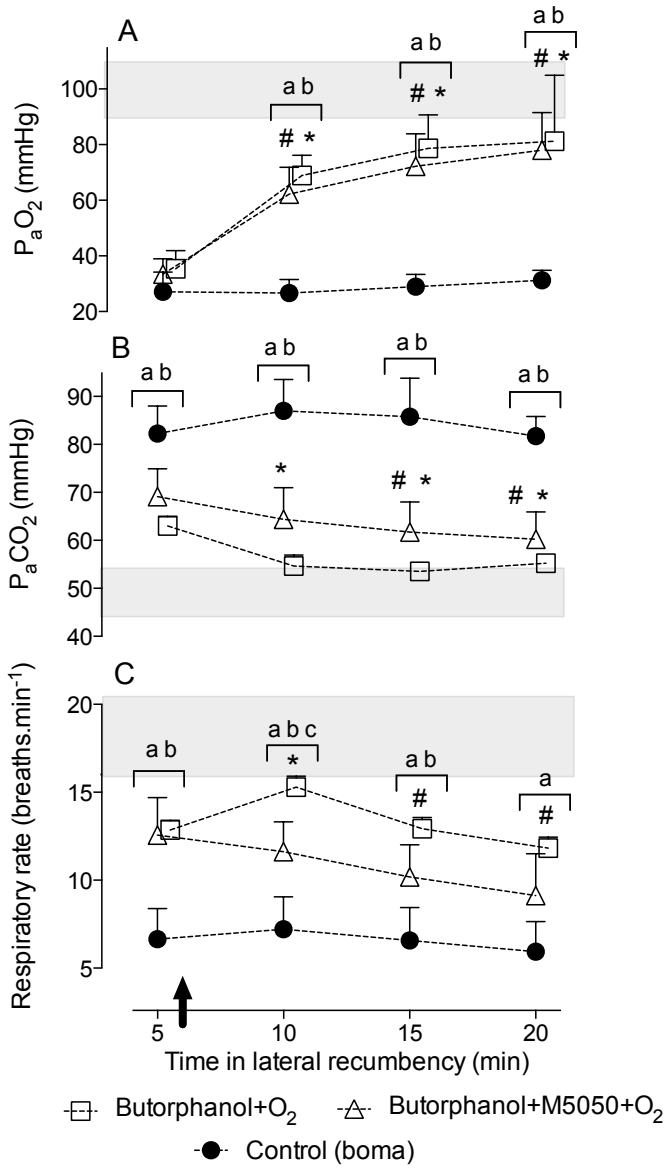


Figure 6.4. Mean and standard deviation of the partial pressure of arterial oxygen (P_aO_2) (A), partial pressure of arterial carbon dioxide (P_aCO_2) (B) and respiratory rate (C) of field-immobilized white rhinoceros given intravenous butorphanol + oxygen insufflation (Butorphanol+O_2, n = 14) or intravenous butorphanol + diprenorphine + oxygen insufflation (Butorphanol+M5050+O_2, n = 8), and boma-immobilized rhinoceros that did not receive supportive treatment (control, n = 8). The black arrow indicates the time at which the partial antagonists were administered and oxygen insufflation initiated. The asterix (*) indicates values in the "butorphanol+O_2" trial that differed from pre-intervention values (5 min) and # indicates values in the "butorphanol+M5050+O_2" trial that differed from pre-intervention values (5 min). The brackets indicate the time points at which values in the rhinoceros under different treatments differed (a = butorphanol+O_2 *versus* control; b = butorphanol+M5050+O_2 *versus* control, c = butorphanol+O_2 versus butorphanol+M5050+O_2). The shaded area represents the range of normal values in standing, unrestrained white rhinoceros (Citino & Bush 2007).

To further improve the practical application of our treatment protocol in white rhinoceros, we conducted another pilot study (not described in chapters 2 to 5) in which we aimed to test the efficacy of administering oxygen in the nostril, rather than the trachea. Nasotracheal intubation may be technically challenging and time-consuming. Placing the oxygen delivery tube to lie within the nostril, rather than the trachea, may be easier and quicker. We withdrew the oxygen delivery tube from the trachea to lie 10 cm within the nostril after 15 minutes of immobilization, and found that P_aO_2 did not change ($p = 0.09$), despite the tube's change in position (Fig. 6.5). Thus nasotracheal intubation does not appear to be necessary to achieve optimum oxygenation in immobilized rhinoceros.

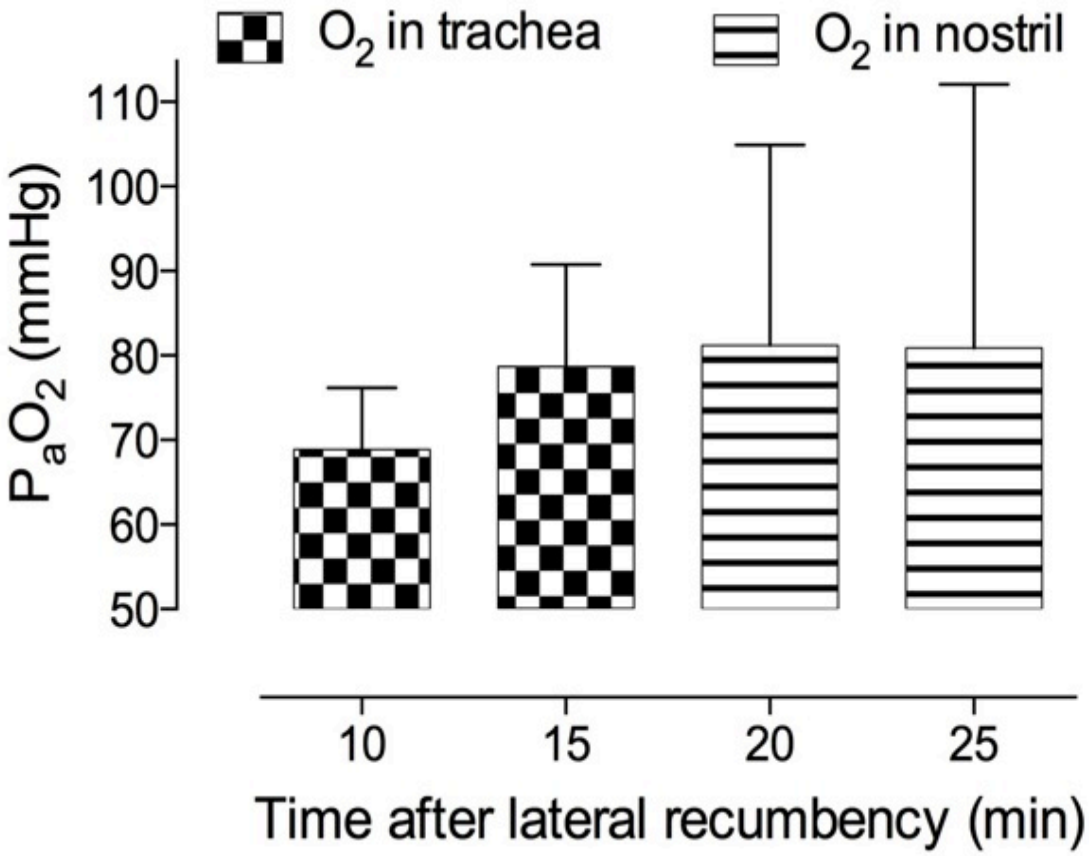
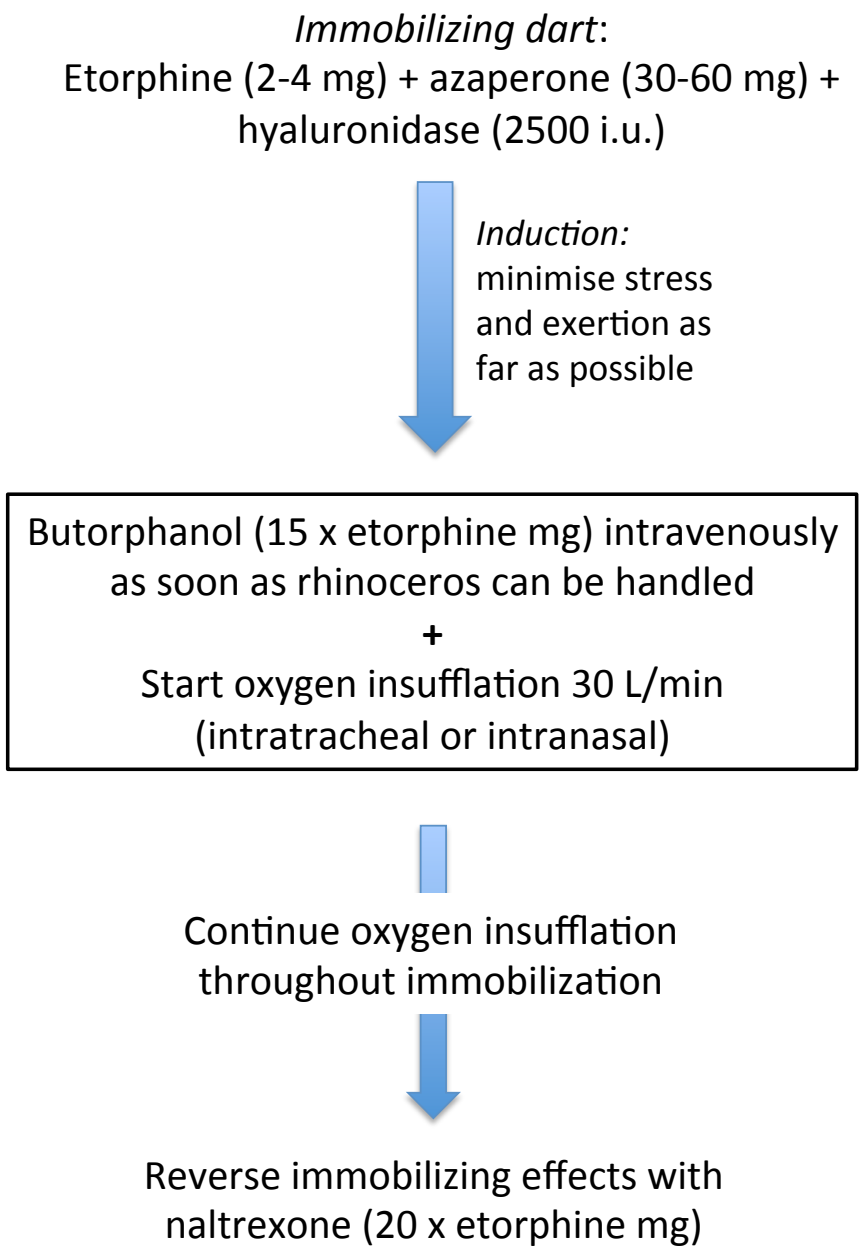


Figure 6.5. The arterial partial pressure of oxygen (P_aO_2) in field-immobilized, recumbent white rhinoceros (n=14). Intravenous butorphanol (15 x etorphine dose) was administered at 5 minutes and oxygen insufflation (30 L/min) was initiated at 5 minutes and continued throughout the immobilization. The oxygen delivery tube was initially placed in the trachea via nasotracheal intubation (O_2 in trachea) and after 15 minutes it was moved to lie 10 cm into the nasal cavity (O_2 in nostril). The partial pressure of arterial oxygen did not change over time ($p = 0.09$), despite a change in position of the oxygen delivery tube.

As a result of my research, my recommended treatment intervention to improve hypoxaemia and hypercapnia in both field and boma etorphine-immobilized white rhinoceros is intravenous butorphanol (or butorphanol + diprenorphine) combined with oxygen insufflation, as detailed in Fig. 6.6.

Figure 6.6: Flow diagram illustrating the recommended immobilization protocol for white rhinoceros.



Although our suggested protocol of administering butorphanol (or butorphanol + diprenorphine) with oxygen to immobilized white rhinoceros is practical and easy to implement, the treatment did not completely resolve the immobilization-induced respiratory compromise. The immobilized rhinoceros remained acidaemic and hypercapnic. Also, the rhinoceros were critically hypoxaemic before butorphanol and oxygen were administered. To prevent, rather than treat opioid-induced hypoxaemia, the respiratory stimulant should be administered in the dart with the immobilizing agent. Butorphanol combined with etorphine in the immobilizing dart has been evaluated in white rhinoceros immobilized in the field (Miller et al. 2013). This immobilization protocol reduced the severity of the initial acidaemia, but the rhinoceros remained hypoxaemic. Also, an experienced capture and animal handling team was required as the majority of rhinoceros remained standing and were livelier after receiving a dart with etorphine combined with butorphanol than etorphine alone (Miller et al. 2013). In addition, it was more difficult to herd the animals by helicopter once the etorphine + butorphanol opioid combination started to take effect, which can be problematic, especially in difficult terrain where the ground crew may be unable to reach the immobilized rhinoceros (Burroughs et al. 2012a). That the rhinoceros remained standing is not surprising, as we have demonstrated that opioid agonist-antagonists decrease the level of immobilization induced by full mu-agonists, such as etorphine (chapter 2). It is therefore necessary to seek alternative ways of reducing opioid-induced respiratory compromise without interfering with the opioid's ability to induce catatonic immobilization.

Apart from opioid agonist-antagonists, wildlife veterinarians use doxapram to treat opioid-induced respiratory compromise (Burroughs et al. 2012b). Doxapram is indicated as a respiratory stimulant for cats and dogs and is often used to stimulate respiration during and after general anaesthesia (Ramsey 2008). This respiratory analeptic is primarily a potassium channel antagonist and exerts its effects centrally, on the brainstem respiratory centres, and peripherally, on carotid and aortic chemoreceptors (Van der Schrier et al. 2014; Yost 2006). Although doxapram causes a profound ventilatory response, its actions are short-lived. Early experiments in dogs revealed that doxapram is metabolized very quickly when administered intravenously (Bruce et al. 1965), which explains the rapid, but transient ventilatory response that I observed in immobilized goats after intravenous administration of doxapram (chapter 5).

Doxapram's profound ventilatory response may also result in hyperventilation, as indicated by the hypocapnia and respiratory alkalosis, which occurred in our goats two minutes after doxapram administration. Indeed, doxapram has been found to cause respiratory alkalosis and hypocapnia in humans (Yost 2006) and neonatal calves (Bleul et al. 2010). Hypocapnia with alkalosis leads to increased haemoglobin affinity for oxygen and thus impairs oxygen delivery at the tissue level (Wilson & Benumof 2005). Another potential negative consequence of hypocapnia is cerebral vasoconstriction with subsequent cerebral hypoxia (Laffey & Kavanagh 2002). At the same time, doxapram may lead to increased cardiac oxygen consumption (Bleul et al. 2010) as a result of the doxapram-induced increased cardiac workload associated with hypertension and increased cardiac output (Van der Schrier et al. 2014), probably as a result of catecholamine release (Yost 2006). Nowadays, the use of doxapram for the improvement of oxygenation in human medicine is therefore considered with caution (Yost 2006). Apart from the cardiovascular alterations, doxapram is known to have a generalized central nervous system stimulatory effect, and in human medicine, doxapram has been used to arouse patients in the post-anaesthetic setting as well as for emergency treatment of drug-induced central nervous system depression (Yost 2006). During wildlife immobilization, the potential arousal effects of doxapram are undesirable.

I therefore searched for a more specific respiratory stimulant with limited side-effects, and a longer duration of action that would be more suitable for use in opioid-immobilized wildlife. Ampakines are a relatively new group of compounds that modulate AMPA receptors, which are critical for respiratory rhythm generation within the brainstem. Certain ampakine molecules have been shown to positively affect drug-induced respiratory depression in both humans and rodents (Oertel et al. 2010; Ren et al. 2006; Ren et al. 2009). However, ampakines had never been tested in an ungulate model, and their effect on etorphine-induced respiratory compromise had not been evaluated. My laboratory goat study (chapter 5) aimed to examine whether ampakines could offer any potential for the treatment of opioid-induced respiratory compromise in ungulates. We found that the administration of the ampakine, CX1942, led to an improvement in respiratory parameters in etorphine-immobilized goats, but the improvement was not as rapid as that observed following the administration of doxapram. However, unlike doxapram, which led to some generalized arousal, CX1942 did not alter the level of

immobilization in the goats. For a more comprehensive study, we should have compared the effects of CX1942 to those of butorphanol in immobilized goats. However, we chose to compare CX1942 to doxapram as doxapram is a specific respiratory stimulant used in a number of animal species, while butorphanol is not classified as a respiratory stimulant, but rather as an opioid agonist-antagonist. The use of butorphanol to improve respiration is relatively specific to etorphine-immobilized herbivores, and in particular, white rhinoceros. The next step would be to do a proper comparative study in white rhinoceros to determine the efficacy of ampakine drugs compared to butorphanol and doxapram.

In a human study, the ampakine CX717 augmented sedation when it was used to prevent alfentanil-induced respiratory depression (Oertel et al. 2010). This ability to increase, rather than reduce sedation may be an additional benefit to the use of ampakines in chemically immobilized wildlife. Also, although we were unable to measure the level of analgesia in our goats, evidence from both human (Oertel et al. 2010) and rat (Ren et al. 2009) studies indicates that ampakines do not reduce opioid analgesia. Another advantage of ampakines is that there is no evidence of them causing hypocapnia when used to treat drug-induced respiratory compromise (Ren et al. 2006; Ren et al. 2015).

Although CX1942 did show some promising results in etorphine-immobilized goats, pharmacological advances with this group of molecules are required before ampakines can be combined in an immobilizing dart to prevent opioid-induced respiratory compromise. Currently, ampakines could potentially be administered to immobilized rhinoceros in place of butorphanol. However, large volumes of the drug would be needed (up to 400 ml in the case of CX1942), which is practically difficult and the required dose would take too long to enter the circulation. Also, a transient tachycardia was recorded in our goats following the administration of CX1942. However, an ampakine-induced tachycardia has not been recorded in rodents or humans. Before administering ampakines to rhinoceros, their efficacy should be proven in wildlife species that develop severe opioid-induced respiratory compromise and respiratory alterations related to capture, similar to those of white rhinoceros. Impala (*Aepyceros melampus*) are wild herbivores that are much smaller and easier to handle than are rhinoceros, and are extremely sensitive to the respiratory depressant effects of opioids. I therefore suggest that impala would offer a good model for further evaluating ampakines in wild species that are particularly sensitive to potent opioids. Thus, until further work is done on the use of ampakines in wildlife, my

recommended treatment intervention for etorphine-immobilized white rhinoceros remains intravenous butorphanol (or butorphanol + diprenorphine) combined with oxygen insufflation (Fig. 6.6).

6.3 Future directions

Our recommended immobilization protocol (Fig. 6.6) corrects etorphine-induced hypoxaemia in immobilized white rhinoceros. However, the suggested protocol does not resolve the etorphine-induced hypercapnia. When the oxygenation in hypoxaemic animals improves, as occurs in immobilized rhinoceros that receive butorphanol with oxygen insufflation, the affinity of carbon dioxide for haemoglobin decreases and carbon dioxide is easily displaced (Haldane effect) (Wilson & Benumof 2005). Thus the overall P_aCO_2 in the blood tends to increase as the P_aO_2 improves (Lian et al. 2014). Also, the ventilatory response to hypoxia is removed with increased P_aO_2, which may exacerbate hypoventilation (Teppema & Dahan 2010; Wilson & Benumof 2005). To reduce the high P_aCO_2 levels, endotracheal intubation and intermittent positive-pressure ventilation would be necessary. A future avenue of research would be to investigate if intermittent positive-pressure ventilation adequately decreases the P_aCO_2 in the immobilized rhinoceros, while also ensuring that the hypoxaemia is corrected. If positive-pressure ventilation were shown to be beneficial, it also would be important to determine if intubation in field-immobilized rhinoceros was feasible and practical. Field immobilization often is complicated by unpredictable events and it is difficult to carry extra equipment or to prolong immobilizations to allow placement of equipment.

Indeed, oxygen cylinders also present a practical obstacle in many field situations, which may prevent veterinarians from administering oxygen, despite its demonstrated benefit in immobilized animals. Conventional oxygen cylinders are cumbersome, prohibited in helicopters, and may be difficult to refill in remote areas. Portable, battery-driven rechargeable oxygen concentrators that extract oxygen from the air may offer a more practical solution to administering oxygen to wildlife immobilized in remote areas (Fahlman et al. 2012). Although the initial purchase of an oxygen concentrator may be expensive, there are no refill costs, and they are small and light with the added advantage of a pulse-dose setting. Pulse-dose oxygen delivery is triggered by the animal's inspiration, thus delivering oxygen only on demand and avoiding wasted oxygen delivered

during expiration as occurs with continuous flow oxygen delivery. However, oxygen concentrators are limited in the maximum amount of oxygen they can deliver. The efficacy of the oxygen concentrator in treating hypoxaemia in immobilized wildlife has been assessed in free-ranging brown bears (*Ursus arctos*) and bighorn sheep (*Ovis canadensis*) (Fahlman et al. 2012). Oxygen delivery via the oxygen concentrator successfully corrected the hypoxaemia in brown bears, but not bighorn sheep (Fahlman et al. 2012). I propose that we test these devices in more species, and specifically rhinoceros, to determine whether this practical method of oxygen delivery will afford the same advantages as we found when oxygen from conventional oxygen cylinders was delivered at a high, continuous flow rate. Perhaps the high oxygen flow rate used in our study is not necessary, but further investigations are needed to determine the minimum required oxygen flow rates in immobilized animals, including white rhinoceros.



Fig. 6.7: Oxygen cylinders may be cumbersome in the field
(Photo credit: Joan Louwrens)

In addition, further research is needed to clarify what physiological disturbances cause worsening respiratory compromise, particularly acidaemia and hypoxaemia, in field- compared to boma-immobilized animals. Indeed, animals in the field may be subjected to a multitude of compounding physiological conditions such as disease and malnutrition. It is imperative that we understand the additional immobilization-induced pathophysiological events in order to adequately manage the body's reaction to different stressors. Once these pathophysiological effects have been clarified, alternative treatment options aimed at correcting the specific physiological conditions, such as pulmonary hypertension, can be implemented (Meyer et al. 2015).

Understanding the physiology of different species under different circumstances may aid in more species-specific treatments for opioid-induced respiratory compromise. It is widely accepted that white rhinoceros are particularly sensitive to opioid-induced respiratory compromise, but the exact opioid pharmacodynamics in white rhinoceros are not fully understood. For example, it is not known why diprenorphine administered to immobilized rhinoceros induces some sedative effects, while even the smallest dose in etorphine-immobilized goats resulted in complete reversal of the immobilization. We know that wildlife species differ in their specific responses to opioids, but how or why such differences occur is yet to be determined. As the wildlife industry in southern Africa is expected to continue to grow, with a number of highly valued species requiring opioid-induced immobilization to facilitate the expansion of the industry, more species-specific studies are needed. Furthermore, accurate comparisons between the available potent opioids, etorphine and thiafentanil, should be carried out in different species to determine the most efficacious opioid with the least side-effects. I have shown that butorphanol with oxygen insufflation treated hypoxaemia in white rhinoceros, but whether that treatment has the same effect in other species, including the closely-related black rhinoceros, remains to be determined. It probably will be important to determine what dose of butorphanol for each individual species will improve breathing without causing unwanted arousal. Indeed, the diversity in responses of different species to opioids is one factor hindering the use of a universal dose of opioid agonist-antagonists to treat opioid-induced respiratory compromise.

Despite years of research, it has not yet been possible to separate the positive effects of mu-opioid receptor activation, such as analgesia and sedation, from the adverse effects

such as respiratory depression. To more accurately separate the pharmacodynamic effects of opioid ligands, we may need to look more towards functional selectivity, rather than receptor selectivity. Opioid receptors appear to display diversity in their activation states, whereby ligands of varied efficacies produce different receptor conformational changes (Boom et al. 2012). Thus, opioids acting on the same receptor may lead to activation of separate intracellular signaling pathways and therefore have different biological outcomes (Boom et al. 2012). In order to antagonise opioid-induced respiratory compromise, but not analgesia, sedation and catatonic immobilization, we need to selectivity antagonise certain conformational states of the mu-opioid receptor. In the future, opioid ligands that bias a specific intracellular pathway, rather than a whole receptor, may provide a more effective route to developing immobilizing and analgesic agents with reduced side-effects, but this advancement in molecular science is yet to be achieved (Shang & Filizola 2015).

Currently, immobilized animals are still subjected to acute pathophysiological events during capture, especially in the period before a veterinarian is able to reach the animal to administer a supportive treatment such as butorphanol with oxygen insufflation. The complications derived from acute adverse physiological effects are not completely understood in wild animals. Follow-up monitoring after opioid-induced immobilization is seldom undertaken, thus the chronic effects of hypoxic cellular damage may be far greater than we realise. We therefore need to judge the success of a capture not solely on the mortality rate, but rather on the long-term survival of the captured animals, and the subsequent reproductive success of the individual (Morkel 1994). I suggest long-term studies that monitor the behaviour and health of captured animals be implemented in order to truly understand the consequences of chemical immobilization.

Together with long-term studies of the health of previously immobilized animals, the physiological effects of transport should be critically evaluated. The growing wildlife industry in southern Africa has led to increasing numbers of game animals being transported, often for many hours. The physiological condition of these animals is not well understood and stressors that occur during both capture and transport may have a significant impact on the ability of the animal to quickly adapt and reproduce in its new environment.

Ultimately we should aim to prevent, rather than treat, opioid-induced respiratory compromise, thus minimizing acute pathophysiological events during capture and transport. Currently, azaperone is routinely co-administered with opioids during chemical immobilization of rhinoceros and other wildlife species. Azaperone enhances the efficacy of opioids, and reduces hypertension through its alpha-1 antagonistic effect, which blocks opioid-induced vasoconstriction (Bowdle 1998; Burroughs et al. 2012b), but unfortunately, azaperone does not positively influence oxygenation (Portas 2004). Ideally, I would like to find a drug that can be co-administered with the immobilizing cocktail to prevent, rather than treat opioid-induced respiratory compromise. The ideal agent should be potent enough so only a small volume is required, it should not interact with other drugs in the dart and it should not interfere with the opioid's ability to induce catatonic immobilization. Thus, opioid-induced respiratory compromise, which is most severe at the beginning of the immobilization period (Meyer et al. 2010), could be prevented rather than treated. Such an approach would significantly reduce the risk of capture-induced complications.

As yet, no such ideal agent exists, but my research has shown that ampakines, such as CX1942, may play an important role in counteracting opioid-induced respiratory compromise in wildlife without causing unwanted arousal. If a highly water-soluble and potent ampakine formulation is developed, prevention and not only reversal of opioid-induced respiratory compromise, as we have demonstrated with CX1942, might be achievable.

6.4 Conclusion

When used appropriately, chemical immobilization can be employed to safely restrain and capture the largest wildlife species, while reducing stress and risk of injuries commonly associated with other methods (Nielsen 1999). However, the ideal situation in which chemically-immobilized animals are completely free from the risk of capture-related morbidity and mortality will require a sophisticated and integrated pharmacological and methodological approach. I believe the results in my thesis have demonstrated ways to improve the respiratory physiology of etorphine-immobilized herbivores, and in particular white rhinoceros. Future efforts should focus on refining our current protocol, for example finding the optimum method of oxygen delivery to reduce hypercapnia and acidaemia. In

addition, I believe ampakines may have a role to play in preventing opioid-induced respiratory compromise, but more soluble and potent formulations are needed before this pharmacological intervention can be properly investigated and utilized in immobilized wildlife.

Although my research has led to many questions, it has also provided critical answers for the advancement of veterinary science. For the first time, different treatment interventions aimed at reversing etorphine-induced respiratory compromise were systematically assessed and compared in white rhinoceros. This trial led to the finding that butorphanol with oxygen insufflation completely corrected, and significantly improved, etorphine-induced hypoxaemia in boma- and field-immobilized white rhinoceros, respectively. We conducted the study in collaboration with veterinarians working in the Kruger National Park, where probably most field rhinoceros immobilizations currently occur, and this new protocol already has been implemented in the Park. Indeed, these advancements will undoubtedly improve the safety of all white rhinoceros immobilizations, and will afford veterinarians the confidence to immobilize injured and ill animals. Moreover, I have opened the eyes of wildlife veterinarians to the world of ampakines, showing that these agents may in future have a significant role to play in preventing opioid-induced respiratory compromise in chemically immobilized herbivores.

As opioids are intricately intertwined in health, conservation and business, their use will continue to increase. The findings displayed in this thesis have taken us a critical step forward in achieving the ideal where clinicians can harness the full beneficial effects of potent opioids without the fear of inducing life-threatening respiratory compromise.

References

Ball RL, Larsen R & Wagman J (2011) Investigating the hemoglobin of white rhinoceros (*Ceratotherium simum simum*) and possible implications for anesthesia. *Proceedings of the 2011 International Elephant and Rhino Conservation and Research Symposium*, Rotterdam. 10-14 Oct 2011, pp. 1497 – 1521.

Baumann R, Mazur G & Braunitzer G (1984) Oxygen binding properties of hemoglobin from the white rhinoceros (β_2-GLU) and the tapir. *Respiration Physiology* 56:1-9.

Bleul U, Bircher B, Jud RS & Kutter APN (2010) Respiratory and cardiovascular effects of doxapram and theophylline for the treatment of asphyxia in neonatal calves. *Theriogenology* 73:612-619.

Boom M, Niesters M, Sarton E, Aarts L, Smith TW & Dahan A (2012) Non-analgesic effects of opioids: opioid-induced respiratory depression. *Current Pharmaceutical Design* 18:5994-6004.

Bowdle TA (1998) Adverse effects of opioid agonists and agonist-antagonists in anaesthesia. *Drug Safety* 19:173-189.

Bowdle TA, Greichen SL, Bjurstrom RL & Schoene RB (1987) Butorphanol improves CO_2 response and ventilation after fentanyl anesthesia. *Anesthesia & Analgesia* 66:517-22.

Bruce RB, Pitts JE, Pinchbeck F & Newman J (1965) Excretion, distribution, and metabolism of doxapram hydrochloride. *Journal of Medicinal Chemisry* 8:157-164.

Burroughs R, Hofmeyr M, Morkel P, Kock MD, Kock R & Meltzer D (2012a). Chemical immobilization – individual species requirements. In: Kock MD, Meltzer D & Burroughs R (eds.), *Chemical and Physical Restraint of Wild Animals* (2nd edition). pp. 223-234, IWVS (Africa), Grayton, South Africa.

Burroughs R, Meltzer D & Morkel P (2012b) Applied Pharmacology. In: Kock MD, Meltzer D & Burroughs R (eds.), *Chemical and Physical Restraint of Wild Animals* (2nd edition). pp. 53-80, IWVS (Africa), Grayton, South Africa.

Bush M, Raath JP & Klein L (2004) Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen. *Journal of the South African Veterinary Association* 75:79-84.

Child BA, Musengezi J, Parent GD & Child GFT (2012) The economics and institutional economics of wildlife on private land in Africa. *Pastoralism: Research, Policy and Practice* 2:18.

Citino SB & Bush M (2007) Reference cardiopulmonary physiologic parameters for standing, unrestrained white rhinoceroses (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 38:375-379.

Dahan A, Aarts L & Smith TW (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 112:226-38.

Dry GG (2015) An analysis of market potential of game meat. Wildlife Farming Conference presentation, 29 August 2015, Tshwane Events Centre, South Africa.

Fahlman Å (2008). Advances in wildlife immobilization and anaesthesia: clinical and physiological evaluation in selected species. Ph.D. thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Fahlman Å, Caulkett N, Arnemo JM, Neuhaus P & Ruckstuhl KE (2012) Efficacy of a portable oxygen concentrator with pulsed delivery for treatment of hypoxemia during anesthesia of wildlife. *Journal of Zoo and Wildlife Medicine* 43:67-76.

Feng Y, He X, Yang Y, Chao D, Lazarus LH & Xia Y (2013) Current research on opioid receptor function. *Current Drug Targets* 13:230-246.

Gao Y & Raj U (2005) Role of veins in regulation of pulmonary circulation. *American Journal of Physiology - Lung Cellular and Molecular Physiology* 288:L213-L226.

Gleed RD & Dobson A (1988) Improvement in arterial oxygen tension with changes in posture in anaesthetized horses. *Research in Veterinary Science* 44:255-259.

Heard DJ, Olsen JH & Stover J (1992) Cardiopulmonary changes associated with chemical immobilization and recumbency in a white rhinoceros (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 23:197-200.

Hyman A & Kadowitz P (1986) Enhancement of α- and β- adrenoceptor responses by elevations in vascular tone in pulmonary circulation. *American Journal of Physiology* 250:H1109 – H1116.

IPCC (2007) Climate Change 2007: Synthesis report. Contribution of Working Groups I, II and II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Pachauri RK & Reisinger A (eds.). IPCC, Geneva, Switzerland.

Janovsky E (2015) Wildlife industry expected to continue to grow. ABSA <http://www.absa.co.za/deployedfiles/Absacoza/PDFs/Commercial/Sector.pdf> [25 January 2016].

Johnson RA and De Morias HA (2012) Respiratory acid-base disorders. In: *Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice* (4th edition). DiBartola SP (ed.). St Louis: Elsevier Saunders, pp. 287-301.

Kadowitz P & Hyman A (1973) Effect of sympathetic nerve stimulation on pulmonary vascular resistance in the dog. *Circulation Research* 32:221-227.

Kay (1997) Responses of African livestock and wild herbivores to drought. *Journal of Arid Environments* 37:683-694.

Kieffer BL (1999) Opioids: First lessons from knockout mice. *Trends in Pharmacological Sciences* 20:19-26.

Kock MD, Morkel P, Atkinson M & Foggin C (1995) Chemical immobilization of free-ranging white rhinoceros (*Ceratotherium simum simum*) in Hwange and Matobo National Parks, Zimbabwe, using combinations of etorphine (M99), fentanyl, xylazine and detomidine. *Journal of Zoo and Wildlife Medicine* 26:207-219.

Kukanich B & Papich MG (2009) Opioid Analgesic Drugs. In: *Veterinary Pharmacology and Therapeutics* (9th edition). Riviere JE & Papich MG (eds.). Blackwell Publishing, Iowa, USA. pp. 301-335.

Laffey JG & Kavanagh BP (2002) Hypocapnia. *New England Journal of Medicine* 347:43-53.

Lewis JW & Husbands SM (2004) The orvinols and related opioids--high affinity ligands with diverse efficacy profiles. *Current Pharmaceutical Design* 10:717-732.

Lian M, Evans AL, Bertelsen MF, Fahlman A, Haga HA, Ericcson G & Arnemo JM (2014) Improvement of arterial oxygenation in free-ranging moose (*Alces alces*) immobilised with etorphine-acepromazine-xylazine. *Acta veterinaria Scandinavia* 56:51-66.

Lindsey PA, Romañach SS & Davies-Mostert HT (2009) The importance of conservancies for enhancing the value of game ranch land for large mammal conservation in southern Africa. *Journal of Zoology* 277:99-105.

MacIntyre PE, Loadsman JA & Scott DA (2011) Opioids, ventilation and acute pain management. *Anaesthetic Intensive Care* 39:545-588.

Marntell S, Nyman G & Hedenstierna G (2005) High inspired oxygen concentrations increase intrapulmonary shunt in anaesthetized horses. *Veterinary Anaesthesia and Analgesia* 32:338-347.

Merriman JE (1955) The role of carbon dioxide in anaesthesia. *Canadian Anaesthetists' Society Journal* 2:273-280.

Meyer LCR, Hetem RS, Fick LG, Matthee A, Mitchell D & Fuller A (2008) Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations. *Journal of the South African Veterinary Association* 79:121-129.

Meyer LCR, Hetem RS, Fick LG, Mitchell D & Fuller A (2010) Effects of serotonin agonists and doxapram on respiratory depression and hypoxemia in etorphine-immobilized impala (*Aepyceros melampus*). *Journal of Wildlife Diseases* 46:514-524.

Meyer LCR, Hetem R, Mitchell D & Fuller A (2015) Hypoxia following etorphine administration in goats (*Capra hircus*) results more from pulmonary hypertension than from hypoventilation. *BMC Veterinary Research* 11:18.

Miller M, Buss P, Joubert J, Mathebula M, Kruger M, Martin L, Hofmeyr M & Olea-Popelka F (2013) Use of butorphanol during immobilization of free-ranging white rhinoceros (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 44: 55-61.

Morkel P (1994) Chemical immobilization of the black rhinoceros (*Diceros bicornis*). Proceedings of a Symposium on "Rhinoceros as Game Ranch Animals", Onderstepoort, Pretoria, 9 & 10 September 1994.

Morkel P, Radcliffe RW, Jago M, du Preez P, Flaminio MJBF, Nydam DV, Taft A, Lain D, Miller M & Gleed RD (2010) Acid-base balance and ventilation during sternal and lateral recumbency in field immobilized black rhinoceros (*Diceros bicornis*) receiving oxygen insufflation: a preliminary report. *Journal of Wildlife Diseases* 46:236-245.

Nielsen L (1999) *Chemical Immobilization of Wild and Exotic Animals*. Iowa State University Press, Ames, Iowa, USA. pp. 3-12.

Nyman G, Funkquist B, Kvart C, Frostell C, Tokics L, Strandberg Å, Lundquist H, Lundh B, Brismar B & Hedenstierna G (1990) Atelectasis causes gas exchange impairment in the anaesthetized horse. *Equine Veterinary Journal* 22:317-324.

Oertel BG, Felden L, Tran PV, Bradshaw MH, Angst MS, Schmidt H, Johnson S, Greer JJ, Geisslinger G, Varney MA & Lötsche J (2010) Selective antagonism of opioid-induced ventilatory depression by an ampakine molecule in humans without loss of opioid analgesia. *Clinical Pharmacology & Therapeutics* 87:204-211.

Okie S (2010) A flood of opioids, a rising tide of deaths. *New England Journal of Medicine* 363:1981-1985.

Pallasch TJ & Gill CJ (1985) Butorphanol and nalbuphine: a pharmacologic comparison. *Oral Surgery, Oral Medicine, and Oral Pathology* 59:15-20.

Pasternak G (2014) Opioids and their receptors: Are we there yet? *Neuropharmacology* 76:198-203.

Portas TJ (2004) A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species. *Australian Veterinary Journal* 82:542-549.

Ramsey I (2008) BSAVA Small Animal Formulary (6th edition), British Small Animal Veterinary Association, Gloucester UK, pp. 111.

Ren J, Poon BY, Tang Y, Funk GD & Greer JJ (2006) Ampakines alleviate respiratory depression in rats. *American Journal of Respiratory and Critical Care Medicine* 174:1384-1391.

Ren J, Ding X, Funk GD & Greer JJ (2009) Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. *Anesthesiology* 110:1364-1370.

Ren J, Ding X & Greer JJ (2015) Ampakines enhance weak endogenous respiratory drive and alleviate apnea in perinatal rats. *American Journal of Respiratory and Critical Care Medicine* 191:704-710.

Rogers PS (1993) Chemical capture of the white rhinoceros *Ceratotherium simum.* In: *The Capture and Care Manual,* McKenzie AA (ed.). Wildlife Decision Support Services and The South African Veterinary Foundation, South Africa pp. 512-528.

Salvi SS (1999) α_1-Adrenergic hypothesis for pulmonary hypertension. *Chest* 115:1708-1719.

Schmidt WK, Tam SW, Schotzberger GS, Smith DH, Clark R & Vernier VG (1985) Nalbuphine. *Drug and Alcohol Dependence* 14:339-362.

Shang Y & Filizola M (2015) Opioid receptors: structural and mechanistic insights into pharmacology and signaling. *European Journal of Pharmacology* 763:206-213.

South African Department of Environmental Affairs <hwww.environment.gov.za> [20 December 2015]

Staffieri F, Bauguier SH, Moate PJ & Driessen B (2009) Pulmonary gas exchange in anesthetised horses mechanically ventilated with oxygen or helium/oxygen mixture. *Equine Veterinary Journal* 41:747-752.

Suter P, Fairley H & Schlobohm R (1975) Shunt, lung volume and perfusion during periods of ventilation with oxygen. *Anesthesiology* 43:617-627.

Swan GE (1993) Drugs used for the immobilization, capture and translocation of wild animals. In: *The Capture and Care Manual,* McKenzie AA (ed.). Wildlife Decision Support Services and The South African Veterinary Foundation, South Africa pp. 3-98.

Teppema LJ & Dahan A (2010) The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. *Physiological Reviews* 90:675-754.

Van der Schrier R, Roozekrans M, Van Velzen M, Dahan A & Niesters M (2014) Opioid-induced respiratory depression: reversal by non-opioid drugs. *F1000 Prime Reports* 6:79.

Vleissentraal (2015) 2015 average game sale prices. <www.vleissentraal.co.za> [23 January 2016]

Volkow ND, Frieden TR, Hyde PS & Cha SS (2014) Medication-assisted therapies – tackling the opioid overdose epidemic. *New England Journal of Medicine* 370:2063-2066.

Wenger S, Boardman W, Buss P, Govender D & Foggin C (2007) The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field-anaesthetized white rhinoceroses (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 38:380-387.

West JB (2012) *Respiratory Physiology the Essentials* (9th edition), Lippincott William & Wilkins, Baltimore, USA.

Wilson WC & Benumof JL (2005) Respiratory physiology and respiratory function during anesthesia. In: *Miller's Anesthesia* (6th edition) Miller RD (ed.). Elsevier Churchill Livingstone, Philadelphia, pp. 679-718.

Yost CS (2006) A new look at the respiratory stimulant doxapram. *CNS Drug Reviews* 12:236-249.

Zucker JR, Neuenfeldt T & Freund PR (1987) Respiratory effects of nalbuphine and butorphanol in anesthetized patients. *Anesthesia & Analgesia* 66:879-881.

REFERENCES

Aaronson PI, Robertson TP, Knock GA, Becker S, Lewis TH, Snetkov V & Ward JPT (2006) Hypoxic pulmonary vasoconstriction: mechanisms and controversies. *The Journal of Physiology* 570:53-58.

Aboab J, Jonson B, Kouatchet A, Taille S, Niklason L & Brochard L (2006) Effect of inspired oxygen fraction on alveolar derecruitment in acute respiratory distress syndrome. *Intensive Care Medicine* 32:1979-1986.

Alford BT, Burkhart RL & Johnson WP (1974) Etorphine and diprenorphine as immobilizing and reversing agents in captive and free-ranging mammals. *Journal of the American Veterinary Medical Association* 164:702-705.

Alibhai SK, Jewell ZC & Towindo SS (2001) Effects of immobilization on fertility in black rhino (*Diceros bicornis*). *Journal of the Zoological Society of London* 253:333-345.

Arai AC, Xia YF & Suzuki E (2004) Modulation of AMPA receptor kinetics differentially infuences synaptic plasticity in the hippocampus. *Neuroscience* 123:1011-1024.

Arnemo JM, Ahlqvist A, Andersen R, Berntsen F, Ericsson G, Odden J, Brunberg S, Segerström P & Swenson JE (2006) Risk of capture-related mortality in large free-ranging mammals: experiences from Scandinavia. *Wildlife Biology* 12:109-113.

Ball RL, Larsen R & Wagman J (2011) Investigating the hemoglobin of white rhinoceros (*Ceratotherium simum simum)* and possible implications for anesthesia. *Proceedings of the 2011 International Elephant and Rhino Conservation and Research Symposium*, Rotterdam, October 10-14, 2011, pp. 1497 – 1521.

Ballanyi K, Lalley PM, Hoch B & Richter DW (1997) cAMP-dependent reversal of opioid- and prostaglandin-mediated depression of the isolated respiratory network in newborn rats. *Journal of Physiology* 504:127-134.

Baumann R, Mazur G & Braunitzer G (1984) Oxygen binding properties of hemoglobin from the white rhinoceros ($|\beta_2$-GLU$|$) and the tapir. *Respiration Physiology* 56:1-9.

Beaver WT & Feise GA (1978) A comparison of the analgesic effect of intramuscular nalbuphine and morphine in patients with postoperative pain. *The Journal of Pharmacology and Experimental Therapeutics* 204:487-496.

Benthuysen JL, Smith NT & Sanford TJ (1986) Physiology of alfentanil-induced rigidity. *Anesthesiology* 64:440-446.

Bianchi AL & Gestreau C (2009) The brainstem respiratory network: an overview of half a century of research. *Respiratory Physiology and Neurobiology* 168:4-12.

Bleul U, Bircher B, Jud RS & Kutter APN (2010) Respiratory and cardiovascular effects of doxapram and theophylline for the treatment of asphyxia in neonatal calves. *Theriogenology* 73:612-619.

Bleul U & Bylang T (2012) Effects of doxapram, prethcamide and lobeline on spirometric, blood gas and acid-base variables in healthy new-born calves. *Veterinary Journal* 194:240-246.

Boardman WSJ, Caraguel CGB, Raath JP & Van Zijll Langhout M (2014) Intravenous butorphanol improves cardiopulmonary parameters in game-ranched white rhinoceroses (*Ceratotherium simum*) immobilized with etorphine and azaperone. *Journal of Wildlife Diseases* 50:849-857.

Bonham AC (1995) Neurotransmitters in the CNS control of breathing. *Respiration Physiology* 101:219-230.

Boom M, Niesters M, Sarton E, Aarts L, Smith TW & Dahan A (2012) Non-analgesic effects of opioids: opioid-induced respiratory depression. *Current Pharmaceutical Design* 18:5994-6004.

Bowdle TA, Greichen SL, Bjurstrom RL & Schoene RB (1987) Butorphanol improves CO_2 response and ventilation after fentanyl anesthesia. *Anesthesia & Analgesia* 66:517-22.

Bowdle TA (1998) Adverse effects of opioid agonists and agonists-antagonists in anaesthesia. *Drug Safety* 19:173-189.

Bruce RB, Pitts JE, Pinchbeck F & Newman J (1965) Excretion, distribution, and metabolism of doxapram hydrochloride. *Journal of Medicinal Chemistry* 8:157-164.

Burroughs R, Hofmeyr M, Morkel P, Kock MD, Kock R & Meltzer D (2012a) Chemical immobilization-individual species requirements. In: *Chemical and Physical Restraint of Wild Animals* (2nd edition). Kock MD & Burroughs R (eds.). IWVS (Africa), Grayton, South Africa. pp. 143-264.

Burroughs R, Meltzer D & Morkel P (2012b) Applied Pharmacology. In: *Chemical and Physical Restraint of Wild Animals* (2nd edition). Kock MD & Burroughs R (eds.). IWVS (Africa), Grayton, South Africa. pp. 53-80.

Burton MD & Kazemi H (2000) Neurotransmitters in central respiratory control. *Respiration Physiology* 122:111-121.

Bush M, Raath JP & Klein L (2004) Severe hypoxaemia in field-anaesthetised white rhinoceros (*Ceratotherium simum*) and effects of using tracheal insufflation of oxygen. *Journal of the South African Veterinary Association* 75:79-84.

Buss P & Meltzer D (2001) Changes in respiratory function following intramuscular administration of etorphine in boer goats (*Capra hircus*). *Journal of the South African Veterinary Assocciation* 72:137-142.

Butelman ER, Winger G, Zernig G & Woods J (1995) Butorphanol: Characterization of agonist and antagonist effects in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics* 272:845-53.

Cattet M, Boulanger J, Stenhouse G, Powell RA & Reynolds-Hogland ML (2008) An evaluation of long-term capture effects in ursids: Implications for wildlife welfare and research. *Journal of Mammalogy* 89:973-990.

Cheney CS & Hattingh J (1987) Effects of chemical immobilization on the blood composition of impala. *Journal of the South African Veterinary Association* 59:13-18.

Child BA, Musengezi J, Parent GD & GFT (2012) The economics and institutional economics of wildlife on private land in Africa. *Pastoralism: Research, Policy and Practice* 2:18.

Citino SB & Bush M (2007) Reference cardiopulmonary physiologic parameters for standing, unrestrained white rhinoceroses (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 38:375-337.

Commiskey S, Fan L-W, Ho IK & Rockhold RW (2005) Butorphanol: effects of a prototypical agonist-antagonist analgesic on κ-opioid receptors. *Journal of Pharmacological Sciences* 98:109-116.

Cornick-Seahorn JL, Mikota SK, Schaeffer DO, Ranglack GS & Boatright SB (1995) Isoflurane anesthesia in a rhinoceros. *Journal of the American Veterinary Medical Association* 206:508-511.

Crumley MN, Hodgson DS & Kreider SE (2012) Effects of tidal volume, ventilator frequency, and oxygen insufflation flow on the fraction of inspired oxygen in cadaveric horse heads attached to a lung model. *American Journal of Veterinary Research* 73:134-139.

Dahan A, Sarton E, Teppema L, Olievier C, Nieuwenhuijs D, Matthes HWD & Kieffer BL (2001) Anesthetic potency and influence of morphine and sevoflurane on respiration in μ-opioid receptor knockout mice. *Anesthesiology* 94:824-832.

Dahan A & Teppema LJ (2003) Influence of anaesthesia and analgesia on the control of breathing. *British Journal of Anaesthesia* 91:40-49.

Dahan A (2007) Novel data on opioid effect on breathing and analgesia. *Seminars in Anesthesia, Perioperative Medicine and Pain* 26:58-64.

Dahan A, Aarts L & Smith TW (2010) Incidence, reversal, and prevention of opioid-induced respiratory depression. *Anesthesiology* 112:226-238.

Daniel M & Ling CM (1972) The effect of an etorphine-acepromazine mixture on the heart rate and blood pressure of the horse. *Veterinary Record* 90:336-339.

DiBartola SP (2006) Metabolic acid-base disorders. In: *Fluid, Electrolyte and Acid-Base Disorders in Small Animal Practice (*3rd edition). DiBartola SP (ed.). Saunders Elsevier, St Louis, Missouri, USA. pp. 251-283.

Dobrenis K, Makman MH & Stefano GB (1995) Occurrence of the opiate alkaloid-selective μ_3 receptor in mammalian microglia, astrocytes and Kupffer cells. *Brain Research* 686:239-248.

Dry GG (2015) An analysis of market potential of game meat. Wildlife Farming Conference presentation, 29 August 2015, Tshwane Events Centre, South Africa.

Dutschmann M, Waki H, Manzke T, Simms AE, Pickering AE, Richter DW & Paton JFR (2009) The potency of different serotonergic agonists in counteracting opioid evoked cardiorespiratory disturbances. *Philosophical Transactions of The Royal Society B* 364:2611-2623.

Dyck MG, Soon W, Baydack RK, Legates DR, Baliunas S, Ball TF & Hancock LO (2007) Polar bears of western Hudson Bay and climate change: Are warming spring air temperatures the "ultimate" survival control factor? *Ecological Complexity* 4:73-84.

El Mallah MK, Pagliardini S, Turner SM, Cerreta AJ, Falk DJ, Byrne BJ, Greer JJ & Fuller DD (2015) Stimulation of respiratory motor output and ventilation in a murine model of Pompe disease by ampakines. *American Journal of Respiratory Cell and Molecular Biology* 53:326-335.

Erlichman JS & Leiter JC (2010) Glia modulation of the extracellular milieu as a factor in central CO_2 chemosensitivity and respiratory control. *Journal of Applied Physiology* 108:1803-1811.

Fahlman Å (2008). Advances in wildlife immobilization and anaesthesia: clinical and physiological evaluation in selected species. Ph.D. thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Fahlman Å, Caulkett N, Arnemo JM, Neuhaus P & Ruckstuhl KE (2012) Efficacy of a portable oxygen concentrator with pulsed delivery for treatment of hypoxemia during anesthesia of wildlife. *Journal of Zoo and Wildlife Medicine* 43:67-76.

Fahlman Å (2014) Oxygen Therapy In: *Zoo Animal and Wildlife Immobilization and Anesthesia* (2nd edition), West G, Heard D & Caulkett N (eds.). John Wiley & Sons, Inc., Iowa, USA. pp. 69-81.

Fahlman Å, Arnemo JM, Pringle J & Nyman G (2014) Oxygen supplementation in anesthetized brown bears (*Ursus arctos*)-How low can you go? *Journal of Wildlife Diseases* 50:574-581.

Feldman JL & Del Negro CA (2006). Looking for inspiration: new perspectives on respiratory rhythm. *Nature Reviews Neuroscience* 7:232.doi:10.1038/nrn1871

Feldman JL, Mitchell GS & Nattie EE (2003) Breathing: rhythmicity, plasticity, chemosensitivity. *Annual Review of Neuroscience* 26:239-266.

Feldman JL, Del Negro CA & Gray PA (2013) Understanding the rhythm of breathing: so near, yet so far. *Annual Review of Physiology* 75:423-452.

Feng Y, He X, Yang Y, Chao D, Lazarus LH & Xia Y (2013) Current research on opioid receptor function. *Current Drug Targets* 13:230-246.

Ferguson LM & Drummond GB (2006) Acute effects of fentanyl on breathing pattern in anaesthetized subjects. *British Journal of Anaesthesia* 96:384-390.

Forster HV, Martino P, Hodges M, Krause K, Bonis J, Davis S & Pan L (2008) The carotid chemoreceptors are a major determinant of ventilator CO_2 sensitivity and of $PaCO_2$ during eupneic breathing. *Advances in Experimental Medicine and Biology* 605:322-326.

Fredholm BB, Battig K, Holmen J, Nehlig A & Zvartau EE (1999) Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews* 51:83-133.

Funk G, Smith J & Feldman J (1993) Generation and transmission of respiratory oscillations in medullary slices: role of excitatory amino acids. *Journal of Neurophysiology* 70:1497-1515.

Ganong WF (ed.) (2005) *Review of Medical Physiology* (22nd edition). McGraw-Hill Companies, Inc., USA. pp. 671-685.

Gao Y & Raj U (2005) Role of veins in regulation of pulmonary circulation. *American Journal of Physiology - Lung Cellular and Molecular Physiology* 288:L213-L226.

Ge Q & Feldman JL (1998) AMPA receptor activation and phosphatase inhibition affect neonatal rat respiratory rhythm generation. *Journal of Physiology* 509:255-266.

Gleed RD & Dobson A (1988) Improvement in arterial oxygen tension with changes in posture in anaesthetized horses. *Research in Veterinary Science* 44:255-259.

Gold SJ, Ambros-Ingerson J, Horowitz JR, Lynch G & Gall CM (1997) Stoichiometries of AMPA receptor subunit mRNAs in rat brain fall into discrete categories. *Journal of Comparative Neurology* 385:491-502.

Golder FJ, Hewitt MM & McLeod JF (2013) Respiratory stimulant drugs in the post-operative setting. *Respiratory Physiology and Neurobiology* 189:395-402.

Greer JJ, Smith JC & Feldman JL (1991) Role of excitatory amino acids in the generation and transmission of respiratory drive in neonatal rat. *Journal of Physiology* 437:727-749.

Grimm KA & Lamont LA (2007) Clinical pharmacology, In: *Zoo Animal and Wildlife Immobilization and Anesthesia*. West G, Heard D & Caulkett N (eds.). Blackwell Publishing Inc. Ames, Iowa, USA. pp. 3-36.

Guenther U, Manzke T, Wrigge H, Dutschmann M, Zinserling J, Putensen C & Hoeft A (2009) The counteraction of opioid-induced ventilatory depression by the serotonin 1_A-agonist 8-OH-DPAT does not antagonize antinociception in rats *in situ* and *in vivo*. *Anesthesia & Analgesia* 108:1169-1176.

Guenther U, Theuerkauf NU, Huse D, Boettcher MF, Wensing G, Putensen C & Hoeft A (2012) Selective 5-HT_{1A}-R-agonist repinotan prevents remifentanil-induced ventilatory depression and prolongs antinociception. *Anesthesiology* 116:56-64.

Gutstein HB & Akil H (2001) Opioid Analgesics. In: *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (10^{th} edition). Hardman JG & Limbirg LE (eds.). McGraw-Hill Medical Publishing Division, USA. pp. 569-619.

Haigh JC (1990) Opioids in zoological medicine. *Journal of Zoo and Wildlife Medicine* 21:391-413.

Haji A, Takeda R & Okazaki M (2000) Neuropharmacology of control of respiratory rhythm and pattern in mature mammals. *Pharmacology & Therapeutics* 86:277-304.

Hajiha M, DuBord MA, Liu H, Horner RL (2009) Opioid receptor mechanisms at the hypoglossal motor pool and effects on tongue muscle activity *in vivo*. *Journal of Physiology* 587:2677-2692.

Harthoorn AM & Bligh J (1965) A new oripavine derivative with potent morphine-like properties for the restraint of the large wild African mammals. *Research in Veterinary Science* 6:290-299.

Harthoorn AM (1966) Restraint of undomesticated animals. *Journal of the American Veterinary Medical Association* 149:875-880.

Harthoorn AM & Young E (1976) Pulmonary hypertension in relationship to acidaemia after maximum forced exercise. A further preliminary report. *Journal of the South African Veterinary Association* 47:187-189.

Hatting J & Knox CM (1994) Arterial blood pressure and blood gas composition of white rhinoceroses under etorphine anaesthesia. *South African Journal of Wildlife Research* 24:12-14.

Haw A, Hofmeyr M, Fuller A, Buss P, Miller M, Fleming G & Meyer L (2014) Butorphanol with oxygen insufflation corrects etorphine-induced hypoxaemia in chemically immobilized white rhinoceros (*Ceratotherium simum*). *BMC Veterinary Research* 10:253.

Heard DJ, Kollias GV, Buss D, Caligiui R & Coniglario J (1990) Comparative cardiovascular effects of intravenous etorphine and carfentanil in domestic goats. *Journal of Zoo and Wildlife Medicine* 21:166-170.

Heard DJ, Olsen H & Stover J (1992) Cardiopulmonary changes associated with chemical immobilization and recumbency in white rhinoceros (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 23:197-200.

Heard DJ, Nichols WW, Buss D & Kollias GV (1996) Comparative cardiopulmonary effects of intramuscularly administered etorphine and carfentanil in goats. *American Journal of Veterinary Research* 57:87-96.

Holden JE, Jeong Y & Forrest JM (2005) The endogenous opioid system and clinical pain management. *AACN Clinical Issues* 16:291-301.

Hoskins PJ & Hanks GW (1991) Opioid agonist-antagonist drugs in acute and chronic pain states. *Drugs* 41:326-344.

Howard P (1989) Hypoxia, almitrine, and peripheral neuropathy. *Thorax* 44:247-250.

Hülsmann S, Oku Y, Zhang W & Richter DW (2000) Metabolic coupling between glia and neurons is necessary for maintaining respiratory activity in transverse medullary slices of neonatal mouse. *European Journal of Neuroscience* 12:856-862.

Hutchinson MR, Northcutt AL, Chao LW, Kearney JJ, Zhang Y, Berkelhammer DL, Loram LC, Rozeske RR, Bland ST, Maier SF, Gleeson TT & Watkins LR (2008) Minocycline suppresses morphine-induced respiratory depression, suppresses morphine-induced reward, and enhances systemic morphine-induced analgesia. *Brain, Behavior, and Immunity* 22:1248-1256.

Huxtable RJ & Schwarz SKW (2001) The isolation of morphine-first principles in science and ethics. *Molecular interventions* 1:189-91.

Hyman A & Kadowitz P (1986) Enhancement of α- and β- adrenoceptor responses by elevations in vascular tone in pulmonary circulation. *American Journal of Physiology* 250:H1109 – H1116.

Inoue M, Kobayashi M, Kozaki S, Zimmer A & Ueda H (1998) Nociceptin/orphanin FQ-induced nociceptive responses through substance P release from peripheral nerve endings in mice. *Proceedings of the National Academy of Sciences of the United States of America* 95:10949-10953.

Inturrisi CE (2002) Clinical pharmacology of opioids for pain. *Clinical Journal of Pain* 13:S1-S11.

IPCC (2007) Climate Change 2007: Synthesis report. Contribution of Working Groups I, II and II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Pachauri RK & Reisinger A (eds.). IPCC, Geneva, Switzerland.

Janczewski WA & Feldman JL (2006) Distinct rhythm generators for inspiration and expiration in the juvenile rat. *Journal of Physiology* 570:407-420.

Janovsky E (2015) Wildlife industry expected to continue to grow. ABSA <http://www.absa.co.za/deployedfiles/Absacoza/PDFs/Commercial/Sector.pdf> [25 January 2016].

Johnson RA & De Morias HA (2012) Respiratory acid-base disorders. In: *Fluid, Electrolyte, and Acid-Base Disorders in Small Animal Practice* (4th edition). DiBartola SP (ed.). St Louis, Elsevier Saunders, pp. 287-301.

Kadowitz P & Hyman A (1973) Effect of sympathetic nerve stimulation on pulmonary vascular resistance in the dog. *Circulation Research* 32:221-227.

Kay (1997) Responses of African livestock and wild herbivores to drought. *Journal of Arid Environments* 37:683-694.

Kellum JA (2007) Disorders of acid-base balance. *Critical Care Medicine* 35:2630-2636.

Kieffer BL (1999) Opioids: first lessons from knock-out mice. *Trends in Pharmacological Sciences* 20:19-26.

Kieffer BL & Gavériaux-Ruff C (2002) Exploring the opioid system by gene knock-out. *Progress in Neurobiology* 66:285-306.

Kilkenny C, Browne WJ, Cuthill IC, Emerson M & Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLoS Biol* 8: e1000412. doi:10.1371/journal.pbio.1000412.

Kim H & Suh Y (2009) Minocycline and neurodegenerative diseases. *Behavioural Brain Research* 196:168-179.

Kisling VN (2001) *Zoo and Aquarium History: Ancient Animal Collections to Zoological Gardens*. CRC Press, Taylor & Francis Group, LLC, USA, pp.1-42.

Knottenbelt MK (1990) Causes of mortality in impala (*Aepyceros melampus*) on 20 game farms in Zimbabwe. *Veterinary Record* 127:47-50.

Kock MD, Clark RK, Franti CE, Jessup DA & Wehausen JD (1987) Effects of capture on biological parameters in free-ranging bighorn (*Ovis Canadensis*): evaluation of normal, stressed and mortality outcomes and documentation of postcapture survival. *Journal of Wildlife Diseases* 23:652-662.

Kock MD, La Grange M & Du Toit R (1990) Chemical immobilization of free-ranging black rhinoceros (*Diceros bicornis*) using combinations of etorphine (M99), fentanyl, and xylazine. *Journal of Zoo and Wildlife Medicine* 21:155-165.

Kock MD, Morkel P, Atkinson M & Foggin C (1995) Chemical immobilization of free-ranging white rhinoceros (*Ceratotherium simum simum*) in Hwange and Matobo National Parks, Zimbabwe, using combinations of etorphine (M99), fentanyl, xylazine and detomidine. *Journal of Zoo and Wildlife Medicine* 26:207-219.

Kukanich B & Papich MG (2009) Opioid Analgesic Drugs. In: *Veterinary Pharmacology and Therapeutics* (9th edition). Riviere JE & Papich MG (eds.). Wiley-Blackwell Publishing, Iowa, USA. pp. 301-335.

Laffey JG & Kavanagh BP (2002) Hypocapnia. *New England Journal of Medicine* 347:43-53.

Lalley PM (2005) D1-dopamine receptor agonists prevent and reverse opiate depression of breathing but not antinociception in the cat. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 289:R45-R51.

Lalley PM (2008) Opioidergic and dopaminergic modulation of respiration. *Respiratory Physiology and Neurobiology* 164:160-167.

Lam A & Newhouse MT (1990) Management of asthma and chronic airflow limitation. Are methylxanthines obsolete? *Chest* 98:44-52.

Larsen DG & Gauthier DA (1989) Effects of capturing pregnant moose and calves on calf survivorship. *The Journal of Wildlife Management* 53:564-567.

Laubie M & Schmitt H (1980) Long-lasting hyperventilation induced by almitrine: evidence for a specific effect on carotid and thoracic chemoreceptors. *European Journal of Pharmacology* 61:125-136.

LeBlanc PH, Eicker SW, Curtis M & Beehler B (1987) Hypertension following etorphine anesthesia in a rhinoceros (*Diceros simus*). *Journal of Zoo Animal Medicine* 18:141-143.

Lewis JW & Husbands SM (2004) The orvinols and related opioids--high affinity ligands with diverse efficacy profiles. *Current Pharmaceutical Design* 10:717-732.

Lian M, Evans AL, Bertelsen MF, Fahlman A, Haga HA, Ericcson G & Arnemo JM (2014) Improvement of arterial oxygenation in free-ranging moose (*Alces alces*) immobilised with etorphine-acepromazine-xylazine. *Acta Veterinaria Scandinavia* 56:51-66.

Lindsey PA, Romañach SS & Davies-Mostert HT (2009) The importance of conservancies for enhancing the value of game ranch land for large mammal conservation in southern Africa. *Journal of Zoology* 277:99-105.

Ling GSF, Spiegel K, Lockhart SH & Pasternak GW (1985) Separation of opioid analgesia from respiratory depression: evidence for different receptor mechanisms. *The Journal of Pharmacology and Experimental Therapeutics* 232:149-155.

Lonergan T, Goodchild AK, Christie MJ & Pilowsky PM (2003) Mu opioid receptors in rat ventral medulla: effects of endomorphine-1 on phrenic nerve activity. *Respiratory Physiology & Neurobiology* 138:165-178.

Lorier AR, Funk GD & Greer JJ (2010) Opiate-induced suppression of rat hypoglossal motoneuron activity and its reversal by ampakine therapy. *PLoS ONE* 5:e8766.

Lötsch J, Skarke C, Schneider A, Hummel T, Geisslinger G (2005) The 5-hydroxy-tryptamine 4 receptor agonist mosapride does not antagonize morphine-induced respiratory depression. *Clinical Pharmacology & Therapeutics* 78:278-87.

Lumb AB (ed.) (2005a) Control of breathing. In: *Nunn's Applied Respiratory Physiology* (6th edition). Elsevier Butterworth Heinemann, Philadelphia, USA. pp. 55-75.

Lumb AB (ed.) (2005b) The pulmonary circulation. In: *Nunn's Applied Respiratory Physiology* (6th edition). Elsevier Butterworth Heinemann, Philadelphia, USA. pp. 92-109.

Lynch G (2004) AMPA receptor modulators as cognitive enhancers. *Current Opinion in Pharmacology* 4:4-11.

Lynch G (2006) Glutamate-based therapeutic approaches: ampakines. *Current Opinion in Pharmacology* 6:82-88.

MacIntyre PE, Loadsman JA & Scott DA (2011) Opioids, ventilation and acute pain management. *Anaesthetic Intensive Care* 39:545-588.

Magnusson L & Spahn DR (2003) New concepts of atelectasis during general anaesthesia. *British Journal of Anaesthesia* 91:61-72.

Magosso E, Ursino M & van Oostrom JH (2004) Opioid-induced respiratory depression: A mathematical model for fentanyl. *IEEE Transactions on Biochemical Engineering* 51:1115-1128.

Manzke T, Ponimaskin EG, Haller M, Dutschmann M, Richter DW, Guenther U & Schwarzacher S (2003) 5-$HT_{4(a)}$ receptors avert opioid-induced breathing depression without loss of analgesia. *Science* 301:226-229.

Marntell S, Nyman G & Hedenstierna G (2005) High inspired oxygen concentrations increase intrapulmonary shunt in anaesthetized horses. *Veterinary Anaesthesia & Analgesia* 32:338-347.

Martin LJ, Blackstone CD, Levey AI, Huganir RL & Price DL (1993) AMPA glutamate receptor subunits are differentially distributed in rat brain. *Neuroscience* 53:327-358.

Mathebula N, Miller M, Buss P, Joubert J, Martin L, Kruger M, Hofmeyr M & Olea-Popelka F (2012) Biochemical values in free-ranging white rhinoceros (*Ceratotherium simum*) in Kruger National Park, South Africa. *Journal of Zoo Wildlife Medicine* 43:530-538.

May CN, Dashwood MR, Whitehead CJ & Mathias CJ (1989) Differential cardiovascular and respiratory responses to central administration of selective opioid agonists in conscious rabbits: correlation with receptor distribution. *British Journal of Pharmacology* 98:903-913.

McDonald J & Lambert DG (2005) Opioid receptors. *Continuing Education in Anaesthesia, Critical Care & Pain* 5:22-25.

McDonell W (1996) Respiratory System. In: *Lumb and Jones' Veterinary Anesthesia* (3rd edition) Thurmon JC, Tranquilli WJ & Benson GJ (eds). Williams and Wilkins, Baltimore. pp. 115-147.

Mellen N, Janczewski WA, Bocchiaro CM & Feldman JL (2003) opioid-induced quantal slowing reveals dual networks for respiratory rhythm generation. *Neuron* 37:821-826.

Meltzer D & Kock N (2012) Stress and capture-related death. In: *Chemical and Physical restrain of wild animals* (2nd edition). Kock MD & Burroughs R (eds.). IWVS (Africa), Greyton, South Africa. pp. 81-88.

Merriman JE (1955) The role of carbon dioxide in anaesthesia. *Canadian Anaesthetist Society Journal* 2:273-280.

Meyer LCR, Fuller A & Mitchell D (2006) Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 290:R405-R413.

Meyer LCR, Hetem RS, Fick LG, Matthee A, Mitchell D & Fuller A (2008) Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations. *Journal of the South African Veterinary Association* 79:121-129.

Meyer LCR, Hetem RS, Fick LG, Mitchell D & Fuller A (2010) Effects of serotonin agonists and doxapram on respiratory depression and hypoxemia in etorphine-immobilized impala (*Aepyceros melampus*). *Journal of Wildlife Diseases* 46:514-524.

Meyer LCR, Hetem R, Mitchell D & Fuller A (2015) Hypoxia following etorphine administration in goats (*Capra hircus*) results more from pulmonary hypertension than from hypoventilation. *BMC Veterinary Research* 11:18.

Mich PM, Wolfe LL, Sirochman TM, Sirochman MA, Davis TR, Lance WR & Miller MW (2008) Evaluation of intramuscular butorphanol, azaperone, and medetomidine and nasal oxygen insufflation for the chemical immobilization of white-tailed deer, *Odocoileus virginianus*. *Journal of Zoo and Wildlife Medicine* 39:480-487.

Miller M, Buss P, Joubert J, Mathebula M, Kruger M, Martin L, Hofmeyr M & Olea-Popelka F (2013) Use of butorphanol during immobilization of free-ranging white rhinoceros (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 44:55-61.

Mironov S & Richter D (2000) Intracellular signalling pathways modulate K_{ATP} channels in inspiratory brainstem neurons and their hypoxic activation: involvement of metabotropic receptors, G-proteins and cytoskeleton. *Brain Research* 853:60-67.

Montandon G, Qin W, Liu H, Ren J, Greer JJ & Horner RL (2011) PreBötzinger complex neurokinin-1 receptor-expressing neurons mediate opioid-induced respiratory depression. *The Journal of Neuroscience* 31:1292-1301.

Morkel P (1994) Chemical immobilization of the black rhinoceros (*Diceros bicornis*). Proceedings of a Symposium on *Rhinoceros as Game Ranch Animals*, Onderstepoort, Pretoria, 9 & 10 September 1994.

Morkel P, Radcliffe RW, Jago M, du Preez P, Flaminio MJBF, Nydam DV, Taft A, Lain D, Miller MM & Gleed RD (2010) Acid-base balance and ventilation during sternal and lateral recumbency in field immobilized black rhinoceros (*Diceros bicornis*) receiving oxygen insufflation: a preliminary report. *Journal of Wildlife Diseases* 46:236-245.

Neumann W, Ericsson G, Dettki H & Arnemo JM (2011) Effect of immobilizations on the activity and space use of female moose (*Alces alces*) *Canadian Journal of Zoology* 89:1013-1018.

Nielsen L (1999) *Chemical Immobilization of Wild and Exotic Animals*. Iowa State University Press, Ames, Iowa, USA. pp. 3-12.

Nishii Y, Okada Y, Yokoba M, Katagiri M, Yanaihara T, Masuda N, Easton PA & Abe T (2008) Aminophylline increases parasternal intercostal muscle activity during hypoxia in humans. *Respiratory Physiology and Neurobiology* 161:69-75.

Nyman G, Funkquist B, Kvart C, Frostell C, Tokics L, Strandberg Å, Lundquist H, Lundh B, Brismar B & Hedenstierna G (1990) Atelectasis causes gas exchange impairment in the anaesthetized horse. *Equine Veterinary Journal* 22:317-324.

Oertel BG, Schneider A, Rohrbacher M, Schmidt H, Tegeder I, Geisslinger G & Lötsch J (2007) The partial 5-hydroxytryptamine 1A receptor agonist buspirone does not antagonize morphine-induced respiratory depression in humans. *Clinical Pharmacology & Therapeutics* 81:59-68.

Oertel BG, Felden L, Tran PV, Bradshaw MH, Angst MS, Schmidt H, Johnson S, Greer JJ, Geisslinger G, Varney MA & Lötsche J (2010) Selective antagonism of opioid-induced ventilatory depression by an ampakine molecule in humans without loss of opioid analgesia. *Clinical Pharmacology & Therapeutics* 87:204-211.

Okie S (2010) A flood of opioids, a rising tide of deaths. *New England Journal of Medicine* 363:1981-1985.

Ostadal B, Ostadalova I & Dhalla NS (1999) Development of cardiac sensitivity to oxygen deficiency: comparative and ontogenetic aspects. *Pharmacological Reviews* 79:635-659.

Pace RW, Mackay DD, Feldman JL & Del Negro CA (2007) Inspiratory bursts in the preBötzinger complex depend on a calcium-activated non-specific cation current linked to glutamate receptors in neonatal mice. *The Journal of Physiology* 582:113-125.

Pallasch TJ & Gill CJ (1985) Butorphanol and nalbuphine: a pharmacologic comparison. *Oral Surgery, Oral Medicine, and Oral Pathology* 59:15-20.

Pasternak G & Pan Y (2011) Mu opioid receptors in pain management. *Acta Anaesthesiologica Taiwanica* 49:21-25.

Pasternak G (2014) Opioids and their receptors: are we there yet? *Neuropharmacology* 76:198-203.

Pattinson KTS (2008) Opioids and the control of respiration. *British Journal of Anaesthesia* 100:747-758.

Picone S, Aufieri R & Paolillo P (2014) Apnea of prematurity: challenges and solutions. *Research and Reports in Neonatology* 4:101-109.

Player I (1967) Translocation of white rhinoceros in South Africa. *Oryx – The International Journal of Conservation* 9:137-150.

Player I (1993) Foreword In: *The Capture and Care Manual*. McKenzie AA (ed.). Wildlife Decision Support Services cc and The South African Veterinary Foundation Pretoria, South Africa. pp. vii-xiii.

Plumb DC (ed.) (2002) *Veterinary Drug Handbook* (4th edition), Iowa State Press, Iowa, USA. pp. 294-295.

Portas TJ (2004) A review of drugs and techniques used for sedation and anaesthesia in captive rhinoceros species. *Australian Veterinary Journal* 82:542-549.

Raath JP (1999) Anesthesia of white rhinoceros. In: *Zoo and Wild Animal Medicine, Current Therapy* (4th edition), Fowler ME & Miller RE (eds.). W.B. Saunders, Philadelphia. pp. 556-571.

Ramirez JM & Richter DW (1996) The neuronal mechanisms of respiratory generation. *Current Opinion in Neurobiology* 6:817-825.

Ramirez JM, Telgkamp P, Elsen FP, Quellmalz UJA & Richter DW (1997) Respiratory rhythm generation in mammals: synaptic and membrane properties. *Respiratory Physiology* 110:71-85.

Ramsey I (2008) BSAVA Small Animal Formulary (6th edition), British Small Animal Veterinary Association, Gloucester UK. pp. 111.

Ren J, Poon BY, Tang Y, Funk GD & Greer JJ (2006) Ampakines alleviate respiratory depression in rats. *American Journal of Respiratory and Critical Care Medicine* 174:1384-1391.

Ren J, Ding X, Funk GD & Greer JJ (2009) Ampakine CX717 protects against fentanyl-induced respiratory depression and lethal apnea in rats. *Anesthesiology* 110:1364-1370.

Ren J, Ding X & Greer JJ (2012) Respiratory depression in rats induced by alcohol and barbiturate and rescue by ampakine CX717. *Journal of Applied Physiology* 113:1004-1011.

Ren J, Lenal F, Yang M, Ding X & Greer JJ (2013) Co-administration of the ampakine CX717 with propofol reduces respiratory depression and fatal apneas. *Anesthesiology* 118:1437-1445.

Ren J, Ding X & Greer JJ (2015) Ampakines enhance weak endogenous respiratory drive and alleviate apnea in perinatal rats. *American Journal of Respiratory and Critical Care Medicine* 191:704-710.

Richter DW, Mironov SL, Büsselberg D, Lalley PM, Bischoff AM & Wilken B (2000) Respiratory rhythm generation: plasticity of a neuronal network. *The Neuroscientist* 6:181-198.

Richter DW & Spyer KM (2001) Studying rhythmogenesis of breathing: comparison of *in vivo* and *in vitro* models. *TRENDS in Neuroscience* 24:464-472.

Richter DW, Manzke T, Wilken B & Ponimaskin E (2003) Serotonin receptors: guardians of stable breathing. *Trends in Molecular Medicine* 9:542-548.

Rogers PS (1993) Chemical capture of the white rhinoceros *Ceratotherium simum.* In: *The Capture and Care Manual*. McKenzie AA (ed.). Wildlife Decision Support Services and The South African Veterinary Foundation, South Africa pp. 512-528.

Romagnoli A & Keats AS (1980) Ceiling effect for respiratory depression by nalbuphine. *Clinical Pharmacology & Therapeutics* 27:478-485.

Roozekrans M, Van der Schrier R, Okkerse P, Hay J, McLeod J & Dahan A (2014) Two studies on reversal of opioid-induced respiratory depression by BK-channel blocker GAL021 in human volunteers. *Anesthesiology* 121:459-68.

Roozekrans M, Olofsen E, Van der Schrier R, van Gerven J, Peng S, McLeod J & Dahan A (2015) Reversal of opioid-induced respiratory depression by BK-channel blocker GAL021: A pharmacokinetic-pharmacodynamic modeling study in healthy volunteers. *Clinical Pharmacology & Therapeutics* 97:641-9.

Roquebert J & Delgoulet C (1988) Cardiovascular effects of etorphine in rats. *Journal of Autonomic Pharmacology* 8:39-43.

Rosenblum A, Marsch LA, Joseph H & Portenoy RK (2008) Opioids and the treatment of chronic pain: controversies, current status, and future directions. *Experimental and Clinical Psychopharmacology* 16:405-416.

Sahibzada N, Ferreira M, Wasserman AM, Taveira-Dasilva AM & Gillis RA (2000) Reversal of morphine-induced apnea in the anesthetized rat by drugs that activate 5-hydroxytryptamine(1A) receptors. *Journal of Pharmacology and Experimental Therapeutics* 292:704-713.

Salvi SS (1999) α_1-Adrenergic hypothesis for pulmonary hypertension. *Chest* 115:1708-1719.

Santiago TV & Edelman EH (1985) Opioids and breathing. *Journal of Applied Physiology* 59:1675-1685.

Santos C, Ferrer M, Roca J, Torres A, Hernandez C & Rodriguez-Roisin R (2000) Pulmonary gas exchange response to oxygen breathing in acute lung injury. *American Journal of Respiratory and Critical Care Medicine* 161:26-31.

Savarie PJ (1976) Pharmacological review of chemicals used for the capture of animals. *Proceedings of the 7th Vertebrate Pest Conference*, 3 January 1976. Paper 41. http://digitalcommons.unl.edu/vpc7/41

Schmidt C, Bellingham MC & Richter DW (1995) Adenosinergic modulation of respiratory neurones and hypoxic responses in the anaesthetized cat. *Journal of Physiology* 483:769-781.

Schmidt WK, Tam SW, Schotzberger GS, Smith DH, Clark R & Vernier VG (1985) Nalbuphine. *Drug and Alcohol Dependence* 14:339-362.

Schultz HD, Li YL & Ding Y (2007) Arterial chemoreceptors and sympathetic nerve activity. Implications for hypertension and heart failure. *Hypertension* 50:6-13.

Schumacher J (2008) Side effects of etorphine and carfentanil in nondomestic hoofstock. In: *Zoo and Wild Animal Medicine* (volume 6). Fowler ME & Miller RE (eds.). Saunders Elsevier, St. Louis, Missouri. pp. 455-461.

Schwarzacker SW, Smith JC & Richter DW (1995) Pre-Bötzinger complex in the cat. *Journal of Neurophysiology* 73:1452-1461.

Shang Y & Filizola M (2015) Opioid receptors: structural and mechanistic insights into pharmacology and signaling. *European Journal of Pharmacology* 763:206-213.

Sharma V, Dutt S, Kumar R, Sharma P, Javed A & Guleria R (2015) OPIOID pharmacology: a review. *International Journal of Scientific Research in Science & Technology* 1:1-11.

Shook JE, Watkins WD & Camporesi EM (1990) Differential roles of opioid receptors in respiration, respiratory disease, and opiate-induced respiratory depression. *American Review of Respiratory Disease* 142:895-909.

Smith JC, Ellenberger HH, Ballanyi K, Richter DW & Feldman JL (1991) Pre-Botzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* 254:726-729.

Smith JC, Abdala APL, Koizumi H, Rybak IA & Paton JFR (2007) Spatial and functional architecture of the mammalian brain stem respiratory network: a hierarchy of three oscillatory mechanisms. *Journal of Neurophysiology* 98:3370-3387.

Smith JC, Abdala APL, Borgmann A, Rybak IA & Paton JFR (2013) Brainstem respiratory networks: building blocks and microcircuits. *Trends in Neuroscience* 36:152-162.

South African Department of Environmental Affairs <hwww.environment.gov.za> [20 December 2015]

Spraker TR (1993) Stress and capture myopathy in artiodactylids. In: *Zoo & Wild Animal Medicine. Current Therapy 3.* Fowler ME (ed.). W.B. Saunders Company, London. pp. 481-488.

Spyer KM & Gourine AV (2009) Chemosensory pathways in the brainstem controlling cardiorespiratory activity. *Philosophical Transactions of the Royal Society B* 364:2603-2610.

Staffieri F, Bauguier SH, Moate PJ & Driessen B (2009) Pulmonary gas exchange in anesthetised horses mechanically ventilated with oxygen or helium/oxygen mixture. *Equine Veterinary Journal* 41:747-752.

Stefano GB, Digenis A, Spector S, Leung MK, Bilfinger TV, Makman MH, Scharrer B & Abumrad NN (1993) Opiate-like substances in an invertebrate, an opiate receptor on invertebrate and human immunocytes, and a role in immunosuppression. *Proceedings of the National Academy of Sciences of the United States of America* 90:11099-11103.

Stein C, Machelska H, Binder W & Schäfer M (2001) Peripheral opioid analgesia. *Current Opinion in Pharmacology* 1:62-65.

Stephen CR & Talton I (1964) Investigation of doxapram as a postanesthetic respiratory stimulant. *Anesthesia & Analgesia* 43:628-640.

Suter P, Fairley H & Schlobohm R (1975) Shunt, lung volume and perfusion during periods of ventilation with oxygen. *Anesthesiology* 43:617-627.

Swan GE (1993) Drugs used for the immobilization, capture and translocation of wild animals. In: *The Capture and Care Manual.* McKenzie AA (ed.). Wildlife Decision Support Services and The South African Veterinary Foundation, South Africa. pp. 3-98.

Takeda S, Eriksson LI, Yamamoto Y, Joensen H, Onimaru H & Lindahl SGE (2001) Opioid action on respiratory neuron activity of the isolated respiratory network in newborn rats. *Anesthesiology* 95:740-749.

Teppema LJ & Dahan A (2010) The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis. *Physiological Reviews* 90:675-754.

Thurmon JC (1990) General clinical considerations for anesthesia of the horse. *Veterinary Clinics of North America: Equine Practice* 6:485-494.

Van der Schrier R, Roozekrans M, Van Velzen M, Dahan A & Niesters M (2014) Opioid-induced respiratory depression: reversal by non-opioid drugs. *F1000 Prime Reports* 6:79.

Van der Schrier R & Roozekrans M (2015) Historical overview of (non-opioid) reversal agends of opioid-induced respiratory depression (OIRD). *Nederlands tijdschrift voor anesthesiology* 28:25-30.

Vankova ME, Weinger MB, Chen D-Y, Bronson B, Motis V & Koob GF (1996) Role of central mu, delta-1, and kappa-1 opioid receptors in opioid-induced muscle rigidity in the rat. *Anesthesiology* 85:574-583.

Vivian JA, DeYoung MB, Sumpter TL, Traynor JR, Lewis JW & Woods JH (1999) Kappa-opioid receptor effects of butorphanol in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics* 290:259-265.

Vleissentraal (2015) 2015 average game sale prices. <www.vleissentraal.co.za> [23 January 2016]

Volkow ND, Frieden TR, Hyde PS & Cha SS (2014) Medication-assisted therapies – tackling the opioid overdose epidemic. *New England Journal of Medicine* 370:2063-2066.

Wenger S, Boardman W, Buss P, Govender D & Foggin C (2007) The cardiopulmonary effects of etorphine, azaperone, detomidine, and butorphanol in field-anaesthetized white rhinoceroses (*Ceratotherium simum*). *Journal of Zoo and Wildlife Medicine* 38:380-387.

Wesensten NJ, Reichardt RM & Balkin TJ (2007) Ampakine (CX717) effects on performance and alertness during simulated night shift work. *Aviation, Space, and Environmental Medicine* 78:937-943.

West JB (2012) *Respiratory Physiology – the essentials* (9th edition). Lippincott Williams & Wilkins, Baltimore, USA.

Whiteside GT, Hummel M, Boulet J, Beyenhof JD, Strenkowski B, Dell John J, Knappenberger T, Maselli H & Koetzner L (2015) Robustness of arterial blood gas analysis for assessment of respiratory safety pharmacology in rats. *Journal of Pharmacological and Toxicological Methods* 78:32-41.

Wilken B, Ramirez JM, Hanefeld F & Richter DW (2000) Aminophylline modulation of the mouse respiratory network changes during postnatal maturation. *Journal of Applied Physiology* 89:2015-2022.

Williams ES & Thorne ET (1996) Exertional myopathy. In: *Noninfectious diseases of wildlife* (2nd edition). Fairbrother A, Locke LL & Hoff GL (eds.). Manson Publishing, London, UK. pp. 181-193.

Wilson WC & Benumof JL (2005) Respiratory physiology and respiratory function during anesthesia. In: *Miller's Anesthesia* (6th edition). Miller RD (ed.). Elsevier Churchill Livingstone, Philadelphia. pp. 679-718.

Yokoba M, Ichikawa T, Takakura A, Ishii N, Kurosaki Y, Yamada Y, Tsukushi T, Masuda N, Easton P & Katagiri M (2015) Aminophylline increases respiratory muscle activity during hypercapnia in humans. *Pulmonary Pharmacology and Therapeutics* 30:96-101.

Yost CS (2006) A new look at the respiratory stimulant doxapram. *CNS Drug Reviews* 12:236-249.

Zöllner C & Stein C (2007) Opioids. *Handbook of Experimental Pharmacology* 177:31-63.

ZooPharm, 2013. *Azaperonetartrate_websiteinfosheet_11_2013*. [online] Available at <www.wildpharm.com/azaperone-tartrate> [21 April 2015].

Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA & Stohler CS (2001) Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science* 293:311-315.

Zucker JR, Neuenfeldt T & Freund PR (1987) Respiratory effects of nalbuphine and butorphanol in anesthetized patients. *Anesthesia & Analgesia* 66:879-81.