THESIS

RESPIRATORY, CARDIOVASCULAR AND METABOLIC EFFECTS OF ETORPHINE, WITH AND WITHOUT BUTORPHANOL, IN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM)*

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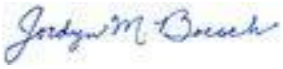
Signature of student: 

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LIST OF ACRONYMS AND ABBREVIATIONS

AC	Adenylyl cyclase (Chapter 1)
AC	Alternating current (Chapter 6)
ADH	Antidiuretic hormone
ADP	Adenosine diphosphate
AEC	Animal Ethics Committee
ANOVA	Analysis of variance
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
AUC	Area under the (response) curve
AUCC	Animal Use and Care Committee
B	Y intercept in the equation for a straight line
B	Beta (Hüfner's factor)
BE	Base excess
BTPS	Body temperature and pressure, saturated with water vapour
Ca^{++}	Calcium
cAMP	Cyclic adenosine monophosphate
CaO_2	Arterial oxygen content
$CaO_2 - C\bar{v}O_2$	Arteriovenous oxygen content difference
Ca_v	Voltage-gated calcium channel
$Cc'O_2$	Pulmonary end-capillary oxygen content
CNA	Central noradrenergic (system)
CNS	Central nervous system
CoA	Coenzyme A
CO_2	Carbon dioxide
CT	Computed tomography
CVLM	Caudal ventrolateral medulla
$C\bar{v}O_2$	Mixed venous oxygen content
δ	Delta (opioid peptide receptor)
DADL	[D-Ala^2, D-Leu^5]-enkephalin
DAGO	D-ala^2-$MePhe^4$-Gly^5-ol-enkephalin
DO_2	Oxygen delivery
DOP	Delta opioid peptide (receptor)
2,3-DPG	2,3-diphosphoglycerate
dSAP	Diastolic systemic arterial pressure
[dysHb]	Concentration of non-oxygen carrying haemoglobins (dyshaemoglobins)
e^-	Electron
ECG	Electrocardiogram
EDTA	Ethylene-diamine-tetra-acetic acid
EIAH	Exercise-induced arterial hypoxaemia
EIPH	Exercise-induced pulmonary haemorrhage
EIT	Electrical impedance tomography
FAD^+	Flavin adenine dinucleotide
F_ECO_2	Expired carbon dioxide fraction
F_EN_2	Expired nitrogen fraction

F_EO_2	Expired oxygen fraction
$F_{\bar{E}}O_2$	Mixed expired oxygen fraction
f_H	Heart rate
F_IN_2	Inspired nitrogen fraction
F_IO_2	Inspired oxygen fraction
Fr	French
f_R	Respiratory rate
FRC	Functional residual capacity
GABA	Gamma (γ)-aminobutyric acid
GDP	Guanosine diphosphate
GPCR	G protein-coupled receptor
GTP	Guanosine triphosphate
H	Hydrogen
H-	Hydride ion
H^+	Proton
Hb	Haemoglobin
[Hb]	Haemoglobin concentration
HCO_3^-	Bicarbonate
$[HCO_3^-]$	Bicarbonate concentration
Hct	Haematocrit
HPLC-ECD	High performance liquid chromatography-electrochemical detection
ID	Identification number
IM	Intramuscularly
IML	Intermediolateral
IU	International units
IUCN	International Union for Conservation of Nature and Natural Resources
IV	Intravenously
J_v	Rate of fluid filtration across a semipermeable membrane
κ	Kappa (opioid peptide receptor)
K^+	Potassium
K_f	Filtration coefficient constant in Starling's equation
KNP	Kruger National Park
KOP	Kappa opioid peptide (receptor)
LDH	Lactate dehydrogenase
LVEDP	Left ventricular end-diastolic pressure
M	Slope in the equation for a straight line
μ	Mu (opioid peptide receptor)
MCHC	Mean corpuscular haemoglobin concentration
MOP	Mu opioid peptide (receptor)
mPAP	Mean pulmonary arterial pressure
mRNA	Messenger ribonucleic acid
mSAP	Mean systemic arterial pressure
N	Nitrogen
NA	Nucleus ambiguus
NAD^+	Nicotinamide adenine dinucleotide
NOP	Nociceptin opioid peptide (receptor)

NTS	Nucleus tractus solitarius
O	Elemental oxygen
ODC	Oxyhaemoglobin dissociation curve
OER	Oxygen extraction ratio
PAC	Pulmonary arterial catheter
$PaCO_2$	Arterial carbon dioxide partial pressure
$P_{A}\text{-}aO_2$	Alveolar-arterial oxygen partial pressure difference
PAG	Periaqueductal gray (matter)
PaO_2	Arterial oxygen partial pressure
P_AO_2	Alveolar oxygen partial pressure
PAOP	Pulmonary arterial occlusion pressure
PAP	Pulmonary arterial pressure
P_B	Barometric pressure
Pc	Pulmonary capillary hydrostatic pressure
$Pc'O_2$	Pulmonary end-capillary oxygen partial pressure
PCO_2	Carbon dioxide partial pressure (general)
PCV	Packed cell volume
P_ECO_2	End-tidal carbon dioxide partial pressure
$P_{\bar{E}}CO_2$	Mixed expired carbon dioxide partial pressure
P_EO_2	End-tidal oxygen partial pressure
Pis	Interstitial hydrostatic pressure
PKA	Protein kinase A
PNS	Peripheral nervous system
PO_2	Oxygen partial pressure (general)
PO_4^{-3}	Phosphate
POM	(Medial) preoptic nucleus
Ptm	Pulmonary transmural pressure
PVN	Paraventricular nucleus
$P\bar{v}O_2$	Mixed venous oxygen partial pressure
PVR	Pulmonary vascular resistance
P_{WV}	Saturated water vapour pressure
Qs/Qt	Shunt fraction (cardiac output shunt/cardiac output total)
Qt	Cardiac output (total)
RER	Respiratory exchange ratio
RCF	Relative centrifugal force
RVLM	Rostral ventrolateral medulla
σ	Sigma (Staverman reflection coefficient of Starling's equation)
SANParks	South African National Parks
SaO_2	Arterial oxyhaemoglobin saturation
SAP	Systemic arterial pressure
SEM	Standard error of the mean
SNS	Sympathetic nervous system
SO_2	Oxyhaemoglobin saturation (general)
SON	Supraoptic nucleus
SpO_2	Oxyhaemoglobin saturation (pulse oximetry)
sSAP	Systolic systemic arterial presure

SV	Stroke volume
$S\bar{v}O_2$	Mixed venous oxyhaemoglobin saturation
SVR	Systemic vascular resistance
SWG	Standard wire gauge
t	Time
τ	Tau (time constant)
T	Temperature
TCA	Tricarboxylic acid (cycle)
T_{lac}	Lactate threshold
UP	University of Pretoria
V_A	Alveolar ventilation
VCO_2	Carbon dioxide production
V_{DPHYS}	Physiological dead space ventilation
V_E	Minute ventilation (minute volume, expired volume)
V_EN_2	Volume of expired nitrogen
V_I	Volume of inspired air
V_IN_2	Volume of inspired nitrogen
VO_2	Oxygen consumption
VO_{2max}	Maximum oxygen consumption
VPC	Ventricular premature depolarisation
V:Q	Ventilation:perfusion (mismatching)
V_T	Tidal volume
Πc	Protein (colloid) osmotic (oncotic) pressure of the plasma
Πg	Protein (colloid) osmotic (oncotic) pressure of the subglycocalyx fluid
7-TMD	7-transmembrane domain
*****	Multiplied by

THESIS EXECUTIVE SUMMARY

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Degree: PhD

This thesis presents data that increases our understanding of the pathophysiological effects of etorphine in the white rhinoceros (*Ceratotherium simum*). This iconic species is poached for its horn throughout southern Africa and must be managed strategically for its protection. Management procedures require chemical immobilisation, and the ultrapotent μ (mu) opioid receptor agonist, etorphine, is one of the few drugs available for rhinoceros immobilisation. However, since etorphine was first introduced in the mid-20th century, veterinarians have observed severe side effects in white rhinoceros during immobilisation, the most serious of which is, arguably, hypoxaemia. Although uncommon, mortalities have occurred.[1] An understanding of the mechanisms by which etorphine causes these derangements will inform efforts to develop preventative or therapeutic strategies that will increase the safety of chemical immobilisation for the white rhinoceros, thus contributing to its conservation. Previous studies have evaluated heart rate (f_H), systemic arterial pressure (SAP), arterial blood gases and acid-base status, minute ventilation (V_E), plasma catecholamine concentrations, metabolic rate, and tremors, among other variables.[2-7] However, a comprehensive understanding of the effects of etorphine requires measurement of pulmonary pressures, cardiac output (Qt),

and mixed venous blood gases and acid-base status as well. To this end, my colleagues and I developed and refined an ultrasound-guided technique for pulmonary arterial catheterisation in the white rhinoceros. In other species, pulmonary arterial catheters (PAC) are inserted through a percutaneous introducer into the jugular vein; the tip is passed through the right heart and into the pulmonary artery. Ultrasound examination suggested that percutaneous access to the jugular vein is impractical in white rhinoceros. However, my colleagues and I found that in this species the introducer can be inserted instead into the linguofacial vein, a branch of the jugular vein, and designed a PAC long enough to reach the pulmonary artery. Pilot testing of a custom-built PAC in boma-habituated white rhinoceros immobilised with etorphine and azaperone, followed by butorphanol intravenously (IV), afforded me the opportunity to assess some of the cardiopulmonary effects of a supplemental, IV bolus of etorphine. I observed an increase in mean pulmonary arterial pressure (mPAP), Qt, and f_H and a decrease in arterial oxygen partial pressure (PaO_2) following an etorphine bolus. Based on normal values in the white rhinoceros and the horse, or values calculated allometrically for the white rhinoceros, these variables were already pathologically increased (mPAP, Qt, f_H) or decreased (PaO_2), and the bolus worsened these physiological derangements. The development and refinement of the pulmonary arterial catheterisation technique, and the data on the effects of an etorphine bolus are detailed in Chapter 2.

After increasing the PAC length to enable measurement of pulmonary arterial occlusion pressure (PAOP), my colleagues and I conducted further research in six boma-habituated, sub-adult, male white rhinoceros. Using a crossover design, each rhinoceros was administered each of two treatments (i.e., two study phases) in random order: etorphine intramuscularly (IM) followed by saline IV (treatment ES), or etorphine IM followed by butorphanol IV (treatment

EB). Once a rhinoceros was positioned in lateral recumbency (time = 0 minutes [t = 0]), 30 minutes was allotted for instrumentation, which included pulmonary and systemic arterial catheterisation and connection to a custom breathing system. Baseline data were collected at t = 30, saline or butorphanol was administered at t = 37, and data were collected again at t = 40 and 50. Chapter 3 presents data from ES and examines changes over time. As reported in previous studies, etorphine produced severe hypoxaemia and hypercapnia, and V_E was lower than normal, expected V_E calculated allometrically.[2] I found that over time, physiological variables remained relatively constant after etorphine administration, with a few exceptions. Mixed venous oxygen partial pressure ($P\bar{v}O_2$), arterial oxygen content (CaO_2), and mixed venous oxygen content ($C\bar{v}O_2$) increased (i.e., improved); tidal volume (V_T) decreased (i.e., worsened), and tremor score and mixed venous lactate concentration decreased (i.e., improved). Oxygen consumption (VO_2) initially decreased (i.e., improved) then increased somewhat. (However, some of these differences lost significance after correction for multiple comparisons; refer to Chapters 3 and 4.) Values for mPAP, PAOP, mean SAP (mSAP), Qt, VO_2, and plasma noradrenaline concentrations were higher than 'normal', assessed by comparison to values measured in other species (or those calculated allometrically), consistent with sympathetic outflow. Chapter 4 examines changes over time in EB and compares ES and EB. I found that butorphanol decreased (i.e., improved) pulmonary pressures, mSAP, Qt, f_H, pulmonary artery and rectal temperatures (T), arterial carbon dioxide partial pressure ($PaCO_2$), VO_2, carbon dioxide production (VCO_2), oxygen extraction ratio (OER), shunt fraction (Qs/Qt), noradrenaline concentration, tremor score, and haemoglobin (Hb) concentration ([Hb]); it also decreased (i.e., worsened) V_T. Butorphanol increased (i.e., improved) pulmonary vascular resistance (PVR), PaO_2, $P\bar{v}O_2$, CaO_2, $C\bar{v}O_2$, minute ventilation body temperature and pressure, saturated with water vapour (V_{EBTPS}), and

respiratory rate (f_R). These differences were either changes from baseline in EB or differences at one or more sampling points between ES and EB. (Again, some of these differences lost significance after correction for multiple comparisons; refer to Chapters 3 and 4.) Stroke volume (SV), alveolar-arterial oxygen partial pressure difference ($P_{A}\text{-}aO_2$), physiological dead space ventilation (V_{DPHYS}), and oxygen delivery (DO_2) in EB were not significantly different from from ES at t = 30, 40 or 50 and did not change significantly within either treatment over time.

We conclude that the hypoxaemia observed in etorphine-immobilised rhinoceros is caused not only by hypoventilation but by venous admixture, which could result from diffusion limitation, ventilation:perfusion (V:Q) mismatching, shunting of blood past completely collapsed alveoli, or some combination of these factors. Although V:Q mismatching due to atelectasis that develops secondary to recumbency likely contributes to some extent, my data support gas diffusion limitation as an important contributing factor. The increase in Qt could have caused gas diffusion limitation by decreasing erythrocyte transit time through pulmonary capillaries, or by increasing pulmonary capillary pressure (Pc) and promoting the development of interstitial pulmonary oedema. A mixed venous hypoxaemia (low $P\bar{v}O_2$ and $C\bar{v}O_2$) exacerbated the systemic arterial hypoxaemia and results from increased VO_2 caused by either skeletal muscle tremors or a generalised increase in metabolic rate. These observations are consistent with the high plasma noradrenaline concentrations measured in the rhinoceros. While sympathetic upregulation might occur secondary to hypoxaemia and hypercapnia, direct stimulation of the hypothalamus or some central noradrenergic (CNA) site by etorphine, causing generalised sympathetic outflow, is supported by research in laboratory animals.[8; 9] In summary, the pathophysiology behind the severe hypoxaemia in etorphine-immobilised rhinoceros is far more complex than some wildlife veterinarians currently realize, and future research into preventative

or therapeutic measures should focus not only on improving V_E and supplementing oxygen, but on mitigating sympathetic outflow.

CHAPTER 1 INTRODUCTION, LITERATURE REVIEW, AND SCOPE OF THE THESIS

Introduction

The southern white rhinoceros (*Ceratotherium simum ssp. simum*) is classified as *near-threatened* by the International Union for Conservation of Nature and Natural Resources (IUCN) due to "the continued and increased poaching threat and increasing illegal demand for horn…".[10] Because of this threat, white rhinoceros in southern Africa must be managed for their protection. Rhinoceros management includes procedures such as translocation or dehorning, which require capture via chemical immobilisation.[11] Chemical immobilisation is also necessary for treating wounds inflicted by other animals and poachers in the field and for undertaking the increasingly complex procedures performed on captive individuals in zoological collections.[12-14] Thus, chemical immobilisation is critical to conservation of the species.

The temperament of rhinoceros and the rugged terrain in which they live necessitates immobilisation using a potent drug with a rapid onset of action that can be delivered intramuscularly (IM) by dart and can be pharmacologically reversed. The ultra-potent μ opioid receptor agonist, etorphine, is one of only a few drugs that fulfils these requirements and has been used for over half a century to chemically immobilise white rhinoceros.[15] Unfortunately, severe and life-threatening hypoxaemia, hypercapnia, acidaemia, tachycardia, systemic arterial hypertension, tremors, and hypermetabolism are ubiquitous in white rhinoceros during chemical immobilisation with etorphine, with hypoxaemia arguably the most serious of these problems.[2-7; 16; 17] Mortalities, though uncommon, do occur, particularly in rhinoceros that are compromised by injury or disease; the death of even a single individual given the ongoing poaching crisis represents a considerable loss for the species.[1] Furthermore, the long-term consequences of these derangements in rhinoceros that survive immobilisation, such as effects on feeding or reproduction, are unknown, a concern that has been voiced by wildlife veterinarians.

Free-ranging rhinoceros are typically darted with etorphine, usually co-administered with the butyrophenone tranquiliser, azaperone, from a helicopter; the opioid agonist-antagonist, butorphanol, is administered intravenously (IV) immediately upon contact with the immobilised rhinoceros.[5; 7] The helicopter triggers a flight response prior to immobilisation that causes the rhinoceros to run.[5] Thus, the physiological derangements observed during immobilisation in the field appear to be an amalgam of exertion, stress, and adverse drug effects. However, white rhinoceros exhibit all of the aforementioned derangements after darting with etorphine in a boma where exertion is minimal.[2-4; 6; 7; 16; 17] This observation suggests that etorphine is primarily responsible for the hypoxaemia and other derangements. Determining which effects are drug-induced, and how etorphine causes these effects, might lead to the development of preventative or therapeutic measures that would increase the safety of immobilisation of the white rhinoceros, thereby contributing to its conservation.

Literature Review

Opioid Pharmacology Overview

Opioids are one of the cornerstones of anaesthetic practice and are virtually indispensable for the chemical immobilisation of rhinoceros and many other wild species. This complex drug class has been reviewed extensively in textbooks and peer-reviewed scientific publications, and a brief summary compiled from these sources is provided here to facilitate the readers' understanding of the pharmacology of the drugs used in this research.[18-22]

The word *opium* (derived from the Greek word *opion*, meaning 'poppy juice') refers to the juice of the opium poppy, *Papaver*.[19] Drugs derived from opium are *opiates*, whereas the term *opioid* refers to all exogenous compounds, whether natural or synthetic, which bind to opioid receptors and produce some degree of morphine-like activity.[19] This term includes the

endogenous opioid peptides (i.e., endorphins, endomorphins, dynorphins, and enkephalins), which are released by opioidergic neurons in both the central and peripheral nervous systems (CNS and PNS) and other cells (e.g., immune cells).[23; 24] They appear to act as the body's natural antinociceptive system; however, they are now also known to play an important role in other functions (discussed below).[19] Opium contains 20 alkaloids that fall into two broad classes: the phenanthrenes and the benzylisoquinolones (which possess no analgesic activity).[19] The major phenanthrenes present in opium are morphine, codeine, and thebaine, the precursor of etorphine (6,14-endoetheno-7-α[2-hydroxy-2 pentyl]-tetrahydro-oripavine, Figure 1-1), a semi-synthetic opioid.[15; 19] Butorphanol contains the phenanthrene core of morphine but is wholly synthetic (Figure 1-2).[19; 25]

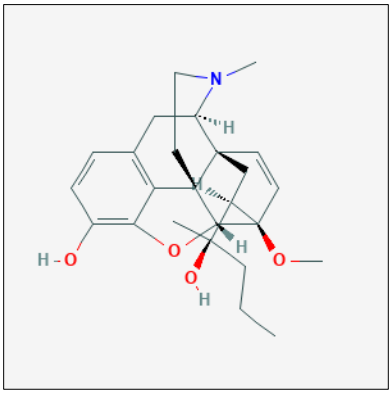

Figure 1-1 The chemical structure of etorphine (https://pubchem.ncbi.nlm.nih.gov/compound/Etorphine).

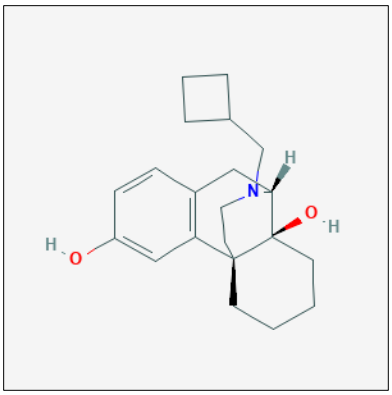

Figure 1-2 The chemical structure of butorphanol. Note the phenanthrene core, similar to morphine and etorphine (http://pubchem.ncbi.nlm.nih.gov/compound/Butorphanol/).

Opioid receptors are located throughout the CNS and PNS, as well as on non-neuronal cells. In the brainstem, opioid receptors are located in central noradrenergic (CNA) sites; the significance of these receptors will be discussed further below. Opioid receptor nomenclature has undergone some change of late.[21] However, in this thesis, the classic Greek letter terminology will be used: μ (mu, now also called 'mu opioid peptide [MOP]' receptor), δ (delta, or 'delta opioid peptide [DOP]' receptor), and κ (kappa, or 'kappa opioid peptide [KOP]' receptor).[21] The fourth member of the opioid receptor family, nociception opioid peptide (NOP) receptor, currently has no known exogenous agonist, and its function is incompletely understood; therefore, it will not be discussed further.[21] This thesis will focus on μ and κ receptors. The distribution of the three classes of opioid receptors differs between species.[26] For instance, of the three, the μ opioid receptor is the most abundant opioid receptor in the cerebral cortex of the horse (71%) but the least abundant receptor in that of the guinea pig (25%).[27] This finding might explain qualitative and quantitative differences among mammals in their responses to opioids.

Opioid receptors are part of the G protein-coupled receptor (GPCR or 7-transmembrane domain [7-TMD] receptor) superfamily (which notably also contains the adrenoceptors).[18] They

consist of a single polypeptide chain comprised of seven hydrophobic cell membrane-spanning α helices connected by short loops.[18] The extracellular loops affect the selectivity of opioids. The intracellular loop contacts the G protein, which consists of α (which can convert guanosine triphosphate [GTP] to guanosine diphosphate [GDP]), β, and γ subunits.[18; 28] When not bound by an opioid, the G protein exists as the αβγ trimer, with GDP occupying the α subunit.[18] When an opioid receptor agonist binds to its receptor, a conformational change in the receptor occurs that causes a high-affinity association between the receptor and G protein (specifically, the $G_{i/o}$ subtypes), and replacement of GDP with GTP.[18] The $G_{i/o}$ protein dissociates into the active forms, $G\alpha_{i/o}$ and Gβγ, which associate with ion channels and enzymes.[22] Binding of an opioid agonist to pre-synaptic opioid receptors (e.g., in the spinal cord dorsal horn) has multiple effects that all result in decreased neurotransmission: 1) Gβγ-mediated inhibition of voltage-gated calcium (Ca^{++}) channels (Ca_v) and other ion channels, which inhibits the release of neurotransmitters (e.g., glutamate, substance P), 2) Gβγ-mediated opening of potassium (K^+) channels, which hyperpolarises the pre-synaptic neuron, and 3) $G\alpha_{i/o}$-mediated inhibition of the enzyme adenylyl cyclase (AC) and therefore, reduction in cyclic adenosine monophosphate (cAMP) concentration, inhibition of protein kinase A (PKA) activity, and, ultimately, inhibition of a number of ion channels.[22] Binding to post-synaptic opioid receptors also opens K^+ channels, which hyperpolarises the neuron. Respiratory depression is caused by Gβγ-mediated opening of the inwardly-rectifying K^+ channel, $K_{ir}3$, in the medulla and pons, the cerebral cortex, thalamus, and amygdala.[22; 29; 30]

Opioids can be classified by their function at the opioid receptor(s). Agonists bind to and activate one or more opioid receptors (e.g., etorphine). Each endogenous opioid peptide class preferentially binds to one of the opioid receptor classes but are agonists at all opioid receptors to

some extent.[18] Antagonists (e.g., naltrexone) bind to but do not activate opioid receptors, preventing the binding of agonists. Agonist-antagonists act as agonists at one opioid receptor class and antagonists at another (e.g., butorphanol).[31] Butorphanol is a κ opioid receptor agonist and partial μ opioid receptor agonist.[31; 32] Butorphanol highlights the difference between affinity (which dictates receptor occupation) and efficacy (which dictates receptor activation).[18] Although butorphanol has a higher μ opioid receptor affinity than morphine, it is only a partial μ opioid receptor agonist because it can only elicit submaximal μ opioid receptor activation, thereby limiting its efficacy, with regard to both positive (e.g., immobilisation, analgesia) and negative (e.g., respiratory depression) effects.[31-34] When administered to an etorphine-immobilised white rhinoceros, it competes with etorphine for μ opioid receptors, antagonising etorphine's effects (hence its classification as an agonist-antagonist). The μ opioid receptor mediates most analgesic effects as well as the catatonia that enables safe handling; unfortunately, it is also responsible for most of the adverse effects, including respiratory depression, gastrointestinal hypomotility, and euphoria.[18; 29; 30] The κ receptor mediates spinal analgesia and sedation but has minimal to no effects on ventilation.[18; 22] Wildlife veterinarians administer butorphanol to manipulate μ opioid receptor binding in an attempt to decrease adverse effects (i.e., respiratory depression) caused by μ opioid receptor agonism while maintaining analgesia and catatonia.

Functional opioid receptor studies that demonstrated differences in relative agonist potency or efficacy at different opioid receptors suggested the existence of subtypes of the three opioid receptors.[35-37] However, to date only one gene for each opioid receptor has been found. It is now known that different opioids acting at the same receptor are capable of activating different signal transduction pathways, a phenomenon known as 'biased agonism'.[21] Opioid receptors are

also known to form homo- and heterodimers with other opioid receptors, and heterodimers or even multimers with other GPCRs, effectively resulting in a different receptor with properties distinct from those of the component receptors.[38-40] Allosteric modulators of opioid receptors may also enhance or inhibit agonist binding and receptor activation.[41] Alternative splicing may account for functional heterogeneity among μ opioid receptors; more than one transcription start site on the μ opioid receptor gene and multiple alternative messenger ribonucleic acid (mRNA) transcripts encoding different proteins have been identified.[21; 42] In summary, opioid pharmacology is extremely complex, with much still to learn. Although etorphine is certainly a full μ opioid receptor agonist, it is likely that its interaction with opioid and other receptors is more complicated than we currently appreciate.[43]

Normal Values for Physiological Variables in White Rhinoceros

Assessment of the effects of anaesthetic drugs in any species requires knowledge of normal values for physiological variables (i.e., values determined in healthy, resting, standing, unstressed, and unmedicated individuals). To my knowledge, Citino and Bush (2007) have published the only such values in the white rhinoceros; Table 1-1 is adapted from this publication and lists normal values for the variables that are measured in this study.[44]

Table 1-1 Physiological variables in 12 healthy, standing, unrestrained, captive white rhinoceros. Data represent five males and seven females ranging in age from 2-31 years old.

	Mean
f_H (beats minute^{-1})	39
f_R (breaths minute^{-1})	19
mSAP (mm Hg)[a]	124

Table 1-1, continued

P_ECO_2 (mm Hg)	45
T (rectal, °C)	36.8
pH	7.391
PaO_2 (mm Hg)[b]	98
$PaCO_2$ (mm Hg)[b]	49

f_H, respiratory rate; f_R, heart rate; mSAP, mean systemic arterial pressure; P_ECO_2, end-tidal carbon dioxide partial pressure; T, temperature; PaO_2, arterial oxygen partial pressure; $PaCO_2$, arterial carbon dioxide partial pressure.

[a] Measured indirectly via oscillometry with a cuff around the tail, corrected to the level of the heart.

[b] Measured in Yulee, Florida, USA, altitude: 4.9 m above sea level (www.weather.gov).

For many variables measured in this study and others, normal values have not been published and would be virtually impossible to obtain in a conscious white rhinoceros. Therefore, in this thesis, when necessary, I use normal values for healthy, standing, unmedicated horses for comparison (e.g., mean pulmonary artery pressure [mPAP]). Because the body mass of the white rhinoceros is substantially greater than that of the horse, variables such as VO_2 should be lower in the white rhinoceros than in the horse; for such variables, I use published allometric equations to calculate expected normal values for these variables in white rhinoceros for comparison to my data. I also indicate, for readers unfamiliar with physiological variables reported in this thesis, when values for these physiological variables improve or worsen.

Etorphine and Rhinoceros

History of Etorphine

Etorphine has been used to capture rhinoceros since the mid-20th century.[15; 45] Before the introduction of etorphine, opioids such as diethylthiambutene and morphine were used to capture

rhinoceros; however, problems such as the large volume of drug required and slow absorption led to the search for alternatives.[15; 46] Investigation of the bridged-ring derivatives of oripavine, a major metabolite of thebaine, led to the synthesis of etorphine (designated as laboratory no. M99 hence its trade name today) in 1963 in Great Britain.[15; 47] As stated above, etorphine is a full μ opioid receptor agonist and is 5,000-10,000 times more potent as an analgesic than morphine.[43]

Early Work with Etorphine

A. M. Harthoorn, the East African physiologist from the Royal College, University of East Africa, and Bligh first reported the use of etorphine in 1965 in *Research in Veterinary Science* in both white and black rhinoceros.[15] Over the next decade or so, an increasing number of manuscripts reported the use of etorphine in both African rhinoceros species.[48-54] In both species, etorphine was used alone, or with other drugs to potentiate its effects.[48-54] The value of combining drugs to improve the quality of immobilisation was recognised early on.[46; 55] Drugs that were co-administered to potentiate etorphine in the early years of rhinoceros immobilisation included fentanyl (an opioid), hyoscine (a CNS depressant, amnesic, and parasympatholytic), acetylpromazine and perphenazine (phenothiazine tranquilisers), azaperone, (a butyrophenone tranquiliser), and phencyclidine (a dissociative anaesthetic).[15; 45; 46; 48-54; 56] Initially, the opioid agonist-antagonists nalorphine, cyprenorphine (M285), and diprenorphine (M5050), or the opioid antagonist naloxone, were used to reverse the effects of etorphine.[15; 45; 48-54; 56]

Recognition of the Adverse Effects of Etorphine

Initially, mortality rate was high, with four of 14 black rhinoceros dying for various reasons in one report; these rhinoceros had been immobilised with 1-3 mg of etorphine as well as hyoscine, acetylpromazine, and, in one animal, phencyclidine.[50] Although mortality apparently improved with time, physiological derangements, which were mentioned in some of the earliest

reports, were still common.[15; 45; 48; 50; 53] Ventilatory depression, manifested as a decrease in respiratory rate (f_R) and depth, was noted, although confirmation of hypercapnia via arterial blood gas analysis was not performed in early rhinoceros field work; heart rates (f_H) that were higher than expected were also observed.[50; 53]

Almost two decades later, two case reports were published which described physiological derangements in two etorphine-immobilised white rhinoceros. One case report described severe systemic arterial hypertension (systolic/diastolic systemic arterial pressure [sSAP/dSAP] = 300/250 mm Hg, measured via an auricular arterial catheter within 15 minutes of recumbency) in a 30-year-old, male white rhinoceros immobilised with 2.8 mg etorphine alone IM; SAP gradually decreased somewhat and stabilised, but the lowest mean SAP (mSAP) recorded was 210 mm Hg, substantially higher than mSAP later recorded in conscious white rhinoceros (Table 1-1).[13] Tachycardia (80-100 beats minute^{-1}) and occasional ventricular premature depolarisations (VPCs) were also noted on the electrocardiogram (ECG).[13] The authors implicated increased sympathetic nervous system (SNS) activity as a possible cause.[13] This report was followed several years later by a case report describing not only hypertension and tachycardia, but tremors, hypoxaemia and, as predicted by earlier reports, hypercapnia in a captive white rhinoceros in a zoological collection in Florida, USA (altitude: 4.9 m above sea level, www.weather.gov) immobilised with etorphine twice over 2 days for reproductive examination.[12] Although the hypertension was not as severe (maximum mSAP = 168 mm Hg) as it was in the first case report, the first measurement, again made via an auricular artery catheter, was recorded 50 minutes after etorphine injection, when the effects of the drug were likely dissipating.[12] The authors proposed the hypertension might have been caused by direct effects of etorphine, by SNS stimulation from hypoxaemia and hypercapnia, by pain, or a combination of

these factors.[12] Nasal oxygen supplementation, which resulted in a peak arterial oxygen partial pressure (PaO_2) of 139 mm Hg (with an arterial carbon dioxide partial pressure [$PaCO_2$] of ~50-60 mm Hg), did not decrease f_H or SAP.[12] Soon after, a small study compared invasive SAP and blood gas partial pressures (measured in the Kruger National Park [KNP], South Africa, precise location and elevation unknown) in white rhinoceros immobilised with either 2 mg of etorphine and 30 mg of fentanyl (n = 6) or 3 mg of etorphine and 25 mg of azaperone (n = 6).[57] Although rigorous statistical analysis of the data were not performed, it appeared that rhinoceros co-administered azaperone with etorphine had lower mSAP, higher PaO_2, and lower $PaCO_2$, despite the larger etorphine dose administered.[57] The lower mSAP in the rhinoceros administered azaperone is consistent with the known vasodilatory properties of azaperone, which are mediated by antagonism of peripheral α_1 adrenoceptors.[58] The lower PaO_2 and higher $PaCO_2$ in the rhinoceros administered fentanyl were attributed to additive effects on ventilation of the two opioids.[57]

A large white rhinoceros capture and dehorning operation was undertaken in Zimbabwe from 1991-1992.[1] A total of 141 rhinoceros were immobilised with either etorphine (1.16 ± 0.28 mg [mean ± SEM], n = 12, calves < 3 years old), etorphine and fentanyl (2.03 ± 0.06 mg and 29.2 ± 0.8 mg, respectively, n = 13, all adults), etorphine and xylazine (4.2 ± 0.11 mg and 123 ± 4.7 mg, respectively, n = 56, all adults), or etorphine and detomidine (an α_2 adrenoceptor agonist, 3.9 ± 0.15 mg and 13.1 ± 0.43 mg, respectively, n = 60, all adults); hyaluronidase (1,500 international units [IU]) was added to all drug combinations.[1] Apparently, this study was the first to describe the use of pulse oximetry in this species. The oximeter probe was placed on the pinna after scraping the skin with a scalpel blade on both sides near the ventral margin of the pinna.[1] Peripheral haemoglobin saturation with oxygen (SpO_2) was measured in 12 rhinoceros, all of

which had been immobilised with etorphine and detomidine; SpO_2 was poor (~40-60%).[1] Although arterial blood gases were not analysed, mucous membrane colour (especially in large bulls and cows) and colour of arterial blood samples were consistent with hypoxaemia.[1] In 1992, partial etorphine antagonism was achieved with IV nalorphine (10-20 mg) or nalbuphine (an opioid agonist-antagonist, 20-40 mg) soon after immobilisation, and SpO_2 subsequently improved by ≥ 20%.[1] However, while f_R was considered adequate (8-12 breaths $minute^{-1}$), the authors considered the rhinoceros to still be hypopneic, and SpO_2 did not normalise.[1] Tachycardia was noted in all groups, although f_H was significantly lower in rhinoceros that had received detomidine; the latter is consistent with the known bradycardic effects of α_2 adrenoceptor agonists in other species.[1] Five mortalities occurred among the first 34 rhinoceros immobilised in 1991 (mortality rate = 7%); the authors thought that three of these could have been prevented by the use of IV nalorphine or nalbuphine, resulting in its routine use in 1992.[1] Paddling, muscle tremors, and shivering were also observed in some rhinoceros; the authors did not state whether or not the five rhinoceros that died were among these.[1] The authors recommended etorphine (3-4 mg for sub-adults to large adult bulls) plus detomidine (12-20 mg) and hyaluronidase (1,500 IU) because of the quality of induction produced.[1] However, because only f_H was measured and not SAP or other cardiovascular variables, conclusions could not be drawn regarding the cardiovascular effects of the combination. The authors also recommended nalorphine (10-20 mg) or nalbuphine (20-40 mg) IV as soon as possible following recumbency to improve breathing.[1] Hyoscine was deemed unnecessary.[1] The authors also suggested the administration of midazolam (10-20 mg), a benzodiazepine and centrally-acting muscle relaxant, IV to improve muscle relaxation and mitigate tremors if needed.[1] Finally, they recommended

antagonism of etorphine with the potent, long-acting opioid antagonist, naltrexone, at the end of a procedure.[1]

Analysis of Blood Gases and Acid-Base Status in Rhinoceros Immobilised with Etorphine

The first description of arterial blood gas and acid-base analysis in etorphine-immobilised white rhinoceros in the field was published in 2004.[59] Twenty-two white rhinoceros were darted from a helicopter with 1.3-4 mg of etorphine, 20-80 mg of azaperone, and 7,500 IU of hyaluronidase.[59] Baseline arterial blood gas analysis (measured in the KNP, South Africa, precise location and elevation unknown) showed hypoxaemia and both respiratory and metabolic acidaemia.[59] Hypertension and tachycardia were also noted.[59] All but four of these rhinoceros were nasotracheally intubated and supplemented with oxygen; nasotracheal intubation and oxygen insufflation improved PaO_2 but not pH or $PaCO_2$.[59] Nalorphine (10-30 mg) administered IV to 12 rhinoceros did not result in marked improvement in $PaCO_2$ or PaO_2.[59]

Over the next decade or so, the administration of butorphanol for respiratory support in etorphine-immobilised white and black rhinoceros gained attention.[60; 61] This practice may have stemmed from publication of case reports in which butorphanol was used either alone or with other drugs, as well as the decreased availability of nalorphine.[16; 62; 63] In 2007, butorphanol was evaluated for the first time in white rhinoceros as a means of reversing the respiratory depression caused by μ opioid receptor agonism by etorphine, without reversing its immobilising and analgesic effects.[61] Thirty-one white rhinoceros of various ages and genders were captured in Malilangwe Wildlife Reserve, Zimbabwe in 2005 (control group, n = 15) and the KNP, South Africa in 2006 (butorphanol group, n = 16); barometric pressure (P_B) reported in this study was 722-742 mm Hg.[61] Rhinoceros in both groups were darted from a helicopter with etorphine, azaperone, detomidine, and hyaluronidase; those in the butorphanol group also received

butorphanol mixed in the same dart (10 mg for calves and 20 mg for sub-adults and adults).[61] In the control group, rhinoceros were positioned in sternal recumbency, whereas in the butorphanol group, they were positioned in lateral recumbency; however, recumbency appears to have changed at an unspecified point during the immobilisations in both groups. In rhinoceros with f_R < 3-4 breaths minute^{-1} and SpO_2 < 80%, nalorphine and diprenorphine were administered IV at unspecified times in relation to arterial blood sampling. Vital parameters were measured and arterial blood was anaerobically sampled from an auricular artery within 10 minutes after immobilisation and again 10 minutes (butorphanol group) or 20 minutes (control group) later.[61] Rectal temperature (T), f_H, and f_R were lower in the control group; however, the control group had been sampled later in the immobilisation period than the butorphanol group had.[61] Consistent with previously published observations, both groups were hypoxaemic and hypercapnic, and had a respiratory as well as a metabolic (lactic) acidaemia.[61] In the control group, PaO_2 was higher and alveolar-arterial oxygen partial pressure difference (P_A-aO_2) was lower (i.e., better) in both body positions than in the butorphanol group.[61] However, this finding can be explained by many factors, including but not limited to 1) the later sampling point in the control group, at which time the effects of etorphine had likely dissipated to some extent, 2) the initial positioning of the control group in sternal recumbency, or 3) the larger number of calves, which had lower body mass than adults or sub-adults, in the control group. Butorphanol administration did not improve $PaCO_2$.[61] Given the problems with study design, it is impossible to draw conclusions about the effects of butorphanol from this publication.

In a retrospective study published in 2013, Miller and colleagues described the use of butorphanol (20 mg for each 1 mg of etorphine) in 40 free-ranging white rhinoceros of various ages and genders in the KNP, South Africa (precise location and elevation unknown).[60] All

rhinoceros were anaesthetised with etorphine (1.5-4.2 mg), azaperone (20-40 mg), and hyaluronidase (1,500 IU) administered by dart from a helicopter.[60] Twenty-two rhinoceros received butorphanol (20 mg for each 1 mg etorphine) IV within 15 minutes after darting (group 1), whereas 18 rhinoceros received butorphanol (20 mg for each 1 mg etorphine) mixed in the dart with the other drugs (group 2).[60] Arterial blood was sampled for analysis ~10 (sample 1) and ~20 (sample 2) minutes after darting in both groups; in group 1 (butorphanol IV), sample 1 was drawn before butorphanol administration.[60] Significantly more rhinoceros in group 2 (butorphanol in dart) than in group 1 (butorphanol IV) remained standing at sample 1 (77.8% vs. 14.0%, respectively).[60] After controlling for the effect of time, body position, and age, rhinoceros in group 2 (butorphanol in dart) had significantly lower (i.e., better) lactate concentration and significantly higher (i.e., better) f_R and pH; more rhinoceros in this group that remained standing, which presumably permitted better ventilation.[60] However, at sample 1, the rhinoceros in group 2 (butorphanol in dart) that became recumbent still had lower (i.e., better) lactate concentration and higher pH than rhinoceros in group 1, suggesting that butorphanol was exerting a beneficial effect.[60] After controlling for the effect of treatment, body position, and age, rhinoceros had significantly lower (i.e., better) f_H, $PaCO_2$, and lactate concentration, and significantly higher (i.e., better) pH at sample 2 vs. 1.[60] These results are consistent with dissipation of the effects of etorphine over time as the drug is metabolised and/or redistributed. When controlling for the effect of treatment, sample period, and age, recumbent rhinoceros had significantly lower (i.e., worse) PaO_2 and higher (i.e., worse) $PaCO_2$ than standing rhinoceros, highlighting the impact of posture on pulmonary function in this species.[60] In recumbent rhinoceros, butorphanol IV decreased (i.e., improved) $PaCO_2$ but did not change PaO_2 between sample periods 1 and 2.[60]

Based on the last two studies in free-ranging white rhinoceros, butorphanol seemed not to cause significant improvement in PaO_2. However, a subsequent study in white rhinoceros that were not darted from a helicopter after a chase, but were rather darted from a vehicle or in bomas, yielded different results. In 2014 at a private game farm in North West Province, South Africa (altitude: 1,333 m above sea level), Boardman and colleagues assessed the effects of butorphanol in 44 game-ranched white rhinoceros of varying ages and genders that had been darted from a vehicle.[64] Vital parameters and, in 20 of the 44 rhinoceros, arterial blood gases and acid-base status, were measured 5 minutes after recumbency; butorphanol (10 mg for each 1 mg etorphine) was then immediately administered IV.[64] Fifteen minutes after recumbency, f_R, pH, and PaO_2 had significantly increased (improved), whereas f_H, SAP, and $PaCO_2$ had significantly decreased (i.e., improved).[64] However, the study lacked a suitable control group.[64]

Recent Rhinoceros Work

Over the last 5 years or so, the quality and complexity of rhinoceros physiological research has increased dramatically. Haw and colleagues (2014) immobilised eight boma-habituated, sub-adult, male white rhinoceros in the KNP, South Africa (altitude: 317 m above sea level), with 2-3 mg etorphine, 30-45 mg azaperone, and 2,500 IU hyaluronidase.[6] Each rhinoceros progressed through each of four treatments at 2 week intervals in random order: 1) butorphanol IV (15 mg for each 1 mg etorphine), 2) butorphanol IV (15 mg for each 1 mg etorphine) plus oxygen insufflation (30 L $minute^{-1}$) via nasotracheal tube, 3) oxygen insufflation (30 L $minute^{-1}$) via nasotracheal tube only, and 4) control (sterile water IV).[6] Baseline data were collected 5 minutes after the rhinoceros was positioned in lateral recumbency (t = 5); the intervention was administered 1 minute later, and data were collected again at t = 10, 15, and 20.[6] In addition to a repeated measures two-way analysis of variance (ANOVA) to test for

differences between PaO_2, $PaCO_2$, and pH at each sampling point and between sampling points within each treatment, areas under the response curves (AUC) were calculated for PaO_2, $PaCO_2$, and pH for the 15 minute period of lateral recumbency to determine the integrated response to each intervention.[6] Consistent with previous studies, rhinoceros were hypoxaemic, hypercapnic, and acidaemic at t = 5, with similar values for PaO_2, $PaCO_2$, and pH across interventions.[6] Butorphanol alone improved (but did not normalise) PaO_2, $PaCO_2$, and pH.[6] Oxygen insufflation exacerbated hypercapnia (resulting in $PaCO_2$ at t = 15 that was ~50% higher than $PaCO_2$ at the same sampling point after control) and acidaemia; although PaO_2 was significantly higher at t = 15 and 20 than PaO_2 at the same sampling points after control, the AUC was not significantly greater.[6] Butorphanol plus oxygen insufflation produced hyperoxaemia; this publication appears to be the first report demonstrating that hypoxaemia could be eliminated during etorphine immobilisation of rhinoceros.[6] However, this combination did not decrease $PaCO_2$ at t = 10, 15, or 20 compared to t = 5, although the AUC was significantly less than the AUC after IV sterile water; arterial blood pH did not increase (i.e., improve).[6] Haw and colleagues (2015) then evaluated the use of butorphanol plus oxygen insufflation in 14 free-ranging, sub-adult, male white rhinoceros immobilised in the KNP (altitude: 317 m above sea level) with 2-3.5 mg etorphine, 30-52.5 mg azaperone, and 2,500 IU hyaluronidase.[5] The data collected in this study were compared with data from boma-immobilised rhinoceros in 1) the control and 2) the butorphanol plus oxygen insufflation treatments from Haw's first study. As in the boma rhinoceros, baseline data were collected 5 minutes (t = 5) after the rhinoceros was positioned in lateral recumbency.[5] At t = 6, butorphanol (15 mg for each 1 mg etorphine) was administered IV, and oxygen insufflation (30 L $minute^{-1}$) was initiated; data were then collected at t = 10, 15, and 20.[5] Baseline PaO_2 was similar to that recorded in the boma rhinoceros; however, unlike in the

boma rhinoceros, the PaO_2 in free-ranging rhinoceros improved but did not normalise.[5] Baseline $PaCO_2$ was high in free-ranging rhinoceros but lower (i.e., better) than that recorded in the boma-habituated rhinoceros; it improved by t = 20 so that only a mild hypercapnia was present.[5] Baseline pH was lower (i.e., worse) than that recorded in the boma rhinoceros but improved; although free-ranging rhinoceros were still acidaemic at t = 20, they were no more acidaemic than the boma rhinoceros were at that sampling point.[5] Lactate concentration was higher in free-ranging than in boma rhinoceros, with 86% having a lactate concentration > 5 mmol L^{-1}, and was positively correlated ($R^2 = 0.42$) with the average speed at which the rhinoceros ran after darting.[5] Free-ranging rhinoceros had lower (i.e., worse) bicarbonate (HCO_3^-) than boma rhinoceros, consistent with titration of protons (H^+) produced in muscle tissue during exertion (from the helicopter chase).[5] Baseline f_H was similar to boma rhinoceros, a surprising finding given the exertion by the free-ranging rhinoceros; after butorphanol, f_H decreased (i.e., improved), but rhinoceros remained tachycardic.[5] However, in both the free-ranging rhinoceros and the boma rhinoceros in the butorphanol-oxygen treatment, f_H was lower (i.e., better) than in the boma rhinoceros in the control treatment.[5] Systemic arterial pressure, measured via a medial auricular artery catheter, also decreased (i.e., improved) after butorphanol.[5]

These two studies provided the opportunity to examine another adverse effect of etorphine immobilisation in the white rhinoceros that has long been recognised but has received little attention: tremors.[1; 7] These tremors involve large muscle groups and range from minor leg and foot movement to whole body and head movement. In 2017, de Lange et al. (2017) published data that had been collected contemporaneously with Haw's data.[7] Every 5 minutes in the boma rhinoceros and every 1 minute in the free-ranging rhinoceros, tremor intensity was assessed in two ways: subjectively, using a scoring system based on visual observations, and

objectively (in the free-ranging rhinoceros only), using an accelerometer attached to the lateral surface of the non-dependent thoracic limb.[7] In addition to the arterial blood samples described in Haw's studies above, venous blood samples were collected for measurement of plasma catecholamine (adrenaline and noradrenaline) concentrations by high-performance liquid chromatography with electrochemical detection (HPLC-ECD).[7] In free-ranging rhinoceros, tremors were most intense immediately after immobilisation and decreased (i.e., improved) after administration of butorphanol IV plus oxygen insufflation.[7] Tremor intensity was not correlated with distance travelled before immobilisation or doses of immobilisation drugs but was correlated negatively with PaO_2 and pH.[7] Both plasma adrenaline and noradrenaline concentrations were positively correlated with tremor intensity.[7] Adrenaline and noradrenaline concentrations were negatively and strongly correlated with PaO_2 and pH, respectively (R^2 = 0.95 and 0.86, respectively).[7] Potassium concentration was correlated positively with tremor intensity and negatively with pH.[7] In boma rhinoceros, butorphanol alone, as well as butorphanol plus oxygen insufflation, decreased tremor intensity, whereas oxygen insufflation alone did not.[7]

A descriptive study to evaluate the cardiorespiratory and blood gas and acid-base effects of extended etorphine-based immobilisation (100 minutes) of white rhinoceros without supplemental oxygen and a single dose of butorphanol (10 mg for each 1 mg etorphine) was conducted by Buss et al. (2015) in the KNP (altitude: 317 m above sea level).[4] Ten boma-habituated white rhinoceros ranging in age from 3-15 years (four males and six females) were darted with standard doses of etorphine (2.5-4 mg) and azaperone (20-40 mg) for their body mass, as well as 5,000 IU of hyaluronidase.[4] Just after the rhinoceros were positioned in sternal recumbency (t = 0), baseline data (rectal T, f_H, f_R, arterial blood samples) were collected; butorphanol was then immediately administered IV.[4] Data were then collected every 10 minutes

for 100 minutes, after which the etorphine was antagonised with naltrexone IV.[4] Typical physiological disturbances were noted before butorphanol administration, including severe hypoxaemia, hypercapnia and respiratory acidaemia, increased P_A-aO_2, tachycardia, and muscle tremors.[4] At 10 and 20 minutes, f_H, P_A-aO_2, f_R, and lactate concentration were lower (i.e., improved) while PaO_2 was higher (i.e., improved), although the rhinoceros remained severely hypoxaemic.[4] During the remaining 80 minutes of data collection, $PaCO_2$ did not improve, but lactate concentration, base excess (BE), HCO_3^-, and pH did.[4] However, the authors admitted that variables such as posture and body mass, which could not be standardised, may have confounded the data.[4]

Because the contribution of the individual drugs to the cardiopulmonary effects observed during immobilisation were still unclear, Buss and colleagues (2016) designed a crossover study in six boma-habituated, sub-adult, male white rhinoceros in the KNP (altitude: 317 m above sea level).[2; 3] Each rhinoceros was administered four treatments in random order: 1) etorphine and hyaluronidase IM, 2) etorphine, azaperone, and hyaluronidase IM, 3) etorphine and hyaluronidase, followed by butorphanol IV, and 4) etorphine, azaperone, and hyaluronidase, followed by butorphanol IV.[2; 3] Data were recorded 10 minutes after recumbency (t = 0) and every 5 minutes thereafter for a total of 25 minutes.[2; 3] In treatments 3 and 4, butorphanol was administered at t = 2.[2; 3] In this study, the cardiovascular effects of etorphine, in isolation, were evaluated for the first time.[3] When immobilised with etorphine only (treatment 1), f_H, and direct SAP declined over the 25 minutes study period.[3] The addition of azaperone to etorphine (treatment 2) resulted in a lower (i.e., improved) f_H (although the rhinoceros were still tachycardic) and SAP.[3] Butorphanol decreased (i.e., improved) f_H and SAP in rhinoceros immobilised with etorphine only (treatment 3).[3] The addition of azaperone to etorphine

(treatment 4) decreased (i.e., improved) SAP, but rhinoceros remained tachycardic; butorphanol decreased (i.e., improved) f_H, but SAP remained unchanged.[3] The investigators also evaluated tremors, ventilation, arterial blood gases and acid-base status, and metabolism in the rhinoceros in this study.[2] Arterial blood gas tensions, tremor intensity, minute ventilation (V_E), and f_R were recorded at each sampling point.[2] Alveolar-arterial oxygen partial pressure difference, expected V_E (calculated allometrically), oxygen consumption (VO_2), and carbon dioxide production (VCO_2) were calculated.[2] Etorphine caused severe hypoxaemia and hypercapnia, and increased (i.e., worsened) P_A-aO_2 and tremors.[2] Butorphanol produced moderate increases (i.e., improvements) in PaO_2 and $PaCO_2$, and decreased (i.e., improved) VO_2 and VCO_2, but did not increase (i.e., improve) V_E.[2] The improvement in PaO_2 was associated with a lower (i.e., better) VO_2, which was associated with a decrease in tremor intensity.[2] This study was the first to demonstrate that a decrease in VO_2, and not an improvement in (alveolar) ventilation (V_A), was responsible for the improvement in PaO_2 after butorphanol administration.

In 2016, van Zijil Langhout and colleagues found that combining midazolam, rather than azaperone, with etorphine for immobilisation of 19 game-ranched white rhinoceros on a private game farm in North West Province, South Africa (altitude: 1,333 m above sea level) resulted in excellent muscle relaxation; only one experienced occasional ear twitches, tremors, and leg movements, while 18 (86%) were completely free of tremors and rigidity, even prior to butorphanol administration IV.[11] Blood gas tensions and acid-base status before and after butorphanol were broadly similar to data collected at the same sampling points by other investigators in similar studies.[6; 11]

Etorphine in the Domestic Horse and Other Species

The domestic horse is a member of the order Perissodactyla (odd-toed ungulates), like the rhinoceros; it also has a broadly similar gastrointestinal tract and cardiopulmonary system. As such, it is possible that observations made on the horse regarding the effects of etorphine, exertion, and posture can be used to inform what is likely to happen in rhinoceros under similar circumstances.

In 1969, a proprietary neuroleptanalgesic combination of etorphine and acepromazine (Large Animal Immobilon) was introduced for use in domestic animals. Hillidge and Lees (1971) were the first to publish the effects of Immobilon in a domestic species, ponies.[65] Rapid IV injection of Immobilon, at the recommended dose, caused increased limb muscle tone, with extension of the thoracic limbs and flexion of the pelvic limbs, as well as full body tremors, in eight of nine ponies; a progressive decrease in muscle tone was sometimes noted after a short time.[65] These clinical manifestations of etorphine immobilisation are very similar to those seen in white rhinoceros but don't usually occur in other species.[1; 7] Tachycardia, arterial hypertension, increased blood glucose concentration, increased haematocrit, and sweating were also recorded, all of which were hypothesised to result from increased SNS activity.[65] Cardiac dysrhythmias, acidaemia, hypercapnia, and hypoxaemia were also noted.[65] Similar adverse effects (e.g., tremors, sweating, tachycardia) were reported in horses administered Immobilon.[66]

A small study in which Immobilon was administered to three ponies and a horse was published in 1972.[67] All animals became tachycardic and hypertensive; as f_H decreased over the period of recumbency, SAP decreased as well.[67] Tachycardia occurred even when a dose of Immobilon too small to cause recumbency was given.[67] Intravenous premedication of two animals with the ß adrenoceptor antagonist, propranolol, mitigated the tachycardia and

hypertension, leading the authors to conclude that these disturbances were the result of drug-induced catecholamine release.[67] A more rapid decline in f_H and SAP followed the administration of diprenorphine (Large Animal Revivon) 30 minutes after Immobilon had been given.[67] The authors stated that this effect demonstrated that the etorphine, and not the acepromazine, was responsible for the tachycardia.[67] A mechanism for the catecholamine release was not determined in this study. Schlarmann and colleagues (1973) speculated that Immobilon liberates the catecholamine, noradrenaline, from post-ganglionic sympathetic neurons rather than systemic release of adrenaline from the adrenal medulla; this finding was based on the speculation that if adrenaline was released, hypotension would likely have occurred because vasodilation of vascular beds controlled by β_2 adrenoceptors would augment that from acepromazine-mediated α_1 antagonism.[68] Such hypotension was not observed, hence noradrenaline release, which has lesser effects at β_2 adrenoceptors, was chosen as the most likely mechanism.[68] However, species-specific differences in Perissodactylae were noted. Whereas IM injection of Immobilon in horses caused an initial excitement phase, in donkeys it did not, and muscle tremors and tachycardia were also absent in donkeys.[69]

Following their initial observations, Lees and Hillidge (1975) conducted further investigations into the cardiopulmonary effects of Immobilon in ponies at the Royal Veterinary College, London (altitude: 11 m above sea level).[70; 71] Consistent with previous work, ponies developed tachycardia, which persisted for at least 30 minutes following Immobilon injection; tachycardia was worse when Immobilon was given IM than when it was given IV.[70] Immobilon increased mSAP from baseline mSAP in the first 5 minutes after injection; however, at 15 and 30 minutes, it had decreased and was similar to baseline mSAP.[70] Mean Qt measured using indocyanine green dye dilution was higher than baseline Qt in all ponies during the 30 minute

data collection period, although there was considerable individual variation with regard to when maximum Qt occurred; as a result, mean Qt was only significantly increased 30 minutes after Immobilon injection.[70] Mean stroke volume (SV, normalised to body mass) was significantly decreased below baseline throughout the entire 30 minute data collection period, presumably because of the decreased ventricular diastolic filling time due to the tachycardia.[70] A statistically insignificant increase above baseline in systemic vascular resistance (SVR) occurred at 5 minutes after injection of Immobilon; however, at 15 and 30 minutes, SVR was significantly lower than baseline SVR.[70] The ponies' respiratory rate and PaO_2 was significantly decreased from baseline PaO_2 throughout the 30 minute data collection period; this hypoxaemia was thought to be a result of a combination of the ponies' lateral recumbency and hypoventilation.[70] However, the authors pointed out that laterally recumbent ponies anaesthetised with metomidate did not become hypoxaemic.[72] Diprenorphine injection increased PaO_2 to nearly 100% of baseline PaO_2.[73] Arterial oxygen content (CaO_2) decreased progressively following Immobilon injection; however, the change at 5 minutes after injection was not statistically significant, primarily because the decrease in PaO_2 was offset by an increased haemoglobin (Hb) concentration ([Hb]).[73] Although a mild respiratory acidaemia was noted, the BE was not changed significantly.[73]

Subsequently, in an attempt to dissect the effects of the individual drugs, Lees et al. (1983) administered only etorphine to ponies, which resulted in profound hypertension (mSAP sometimes > 300 mm Hg) that persisted until diprenorphine administration.[74] The increase in haematocrit following etorphine administration was also greater and more persistent than the increase following Immobilon administration, possibly due to splenic engorgement with erythrocytes by the acepromazine in Immobilon.[74] The increase in f_H was similar when etorphine

and Immobilon were given.[74] However, Bogan et al. (1978) found that f_H increased more when Immobilon was given compared to etorphine alone, and that the increase in f_H and SAP following etorphine administration was accompanied by a rise in noradrenaline concentration from a mean of 0.73 pmol L^{-1} to 4.04 pmol L^{-1} 5 minutes after etorphine was given.[75]

Pulmonary arterial pressure (PAP) and pulmonary arterial occlusion pressure (PAOP) have not been measured in the horse following etorphine administration; however, there is speculation that pulmonary hypertension occurs when potent opioids are used for immobilisation of horses. Pulmonary pressures have, however, been measured in the domestic goat before and after etorphine administration.[76] In that study, conducted in Johannesburg, South Africa (altitude: 1,753 m above sea level), etorphine administration caused hypercapnia and hypoxaemia, with an increased P_A-aO_2, indicating that hypoventilation alone could not be solely responsible for the hypoxaemia.[76] An increase in PAP (pulmonary arterial hypertension) was observed as well, leading the authors to speculate that hypoxaemia could have resulted from increased capillary hydrostatic pressure (Pc) and increased extravasation of fluid from pulmonary capillaries into the interstitium.[76] If this were to exceed the rate of removal of interstitial fluid by lymphatic vessels, then interstitial pulmonary oedema would ensue, which in turn, would cause limitation of oxygen diffusion from the alveolar gas into the capillary blood, and hypoxaemia.[76] Because pulmonary vascular resistance (PVR) also increased, pulmonary vasoconstriction was implicated as the cause of the pulmonary hypertension.[76] However, the cause of this vasoconstriction could not be identified.[76] Although it could have been due to sympathetic outflow, no other signs of generalised SNS activation, such as tachycardia or systemic arterial hypertension, were seen in these goats, at variance to observations in horses.[76]

Central Opioid Receptors, the Central Noradrenergic System, and Catecholamines

The literature indicates that the SNS might play a crucial role in the pathophysiological effects of etorphine in both horses and white rhinoceros. The influence of opioids on autonomic function was recognised in the early 19th century, and a large body of literature on the subject produced over the last 200 years provides insight into how etorphine might cause sympathetic outflow.[8]

A basic understanding of the anatomy of the central noradrenergic (CNA) system, which has been well-characterised, is necessary before delving into the effects of opioids on autonomic function.[77-79] The brain is broadly divided, from cranial to caudal, into the forebrain (which consists of the telencephalon and diencephalon), midbrain, and hindbrain (which consists of the pons, cerebellum, and medulla oblongata). The brainstem consists of the midbrain, pons, and medulla oblongata and contains the most important components of the CNA: 1) the locus coeruleus (LC) and subcoeruleus (located in the pons) and 2) the medulla.[79] Two sites in the medulla that may play an important role in the pathophysiological effects of etorphine are 1) the rostral and caudal ventrolateral medulla (RVLM and CVLM) and 2) the nucleus of the solitary tract (NTS). Neurons that ascend and descend from these CNA sites innervate almost every other part of the CNS, including the cerebral cortex, limbic forebrain structures, thalamus, hypothalamus, cerebellum, medulla, and spinal cord.[79]

The hypothalamus, the primary centre governing neuroendocrine, autonomic, and homeostatic functions, is part of the diencephalon of the forebrain and consists of three distinct regions; the most anterior (rostral) is the anterior (rostral) supraoptic region.[77] The hypothalamus is also divided into the medial, cell-rich zone, which is organised into discrete nuclei, and the lateral zone, which cannot be organised into discrete nuclei but contains axons that convey

information into and out of the medial zone.[77] Two prominent nuclei in the anterior supraoptic region are the supraoptic nucleus (SON) and the paraventricular nucleus (PVN), both of which are highly vascularised.[77] Their axons travel down into the posterior pituitary gland (simply an extension of the hypothalamus), where they release vasopressin (antidiuretic hormone [ADH]) and oxytocin into the bloodstream.[77] The PVN is the larger of the two and is one of the most important autonomic control centres in the brain.[77; 80] These nuclei communicate with autonomic and other regions of the brainstem and spinal cord; these connections can be afferent or efferent and will be discussed in greater detail below.[77; 81] The infundibular region at the base of the hypothalamus, from which the pituitary stalk arises, contains the portal vessels that allow humoral communication between the hypothalamus and the anterior pituitary gland.[77] These vessels lack the tight junctions of the blood-brain barrier and thus, the hypothalamus could theoretically be rapidly exposed to a substance carried by the systemic circulation to these vessels.[77]

Monoamines are one of two classes of amine neurotransmitters and are characterised by a single amine group derived from an amino acid; the catecholamines (noradrenaline, adrenaline, and dopamine) are one of the groups of monoamines.[82] A large number of monoaminergic neurons in the medulla oblongata project directly to the hypothalamus; for instance, afferent noradrenergic neurons from 1) the RVLM and CVLM and 2) the NTS travel directly to the PVN, SON, and the medial preoptic nucleus (POM) of the anterior hypothalamus.[77] The RVLM projects directly to the cell bodies of the preganglionic sympathetic neurons in the intermediolateral (IML) cell column of the thoracolumbar spinal cord and is therefore the final site of output to these neurons.[83; 84] The NTS is the site of input from peripheral baroreceptors in the aorta and carotid bodies and inhibits the RVLM to decrease f_H in response to an increase in

SAP; the NTS will be discussed further below.[77; 85] Efferent neurons from the SON, PVN, and POM of the hypothalamus travel to the VLM and NTS.[77; 86] Stimulation of these hypothalamic areas increases RVLM activity and sympathetic outflow.[81] Although the PVN is best known for secreting oxytocin and ADH as described above, it also secretes endogenous opioid peptides (e.g., dynorphin, enkephalin) to communicate with the VLM and NTS.[77; 81] Preganglionic sympathetic neurons in the IML column also receive direct input from brain regions such as the PVN of the hypothalamus.[87] Preganglionic sympathetic neurons synapse with postganglionic sympathetic neurons in various ganglia in the body; they also synapse with the adrenal medulla.[88] The postganglionic neurons communicate with the heart, blood vessels, and other tissues by releasing noradrenaline, while the chromaffin cells of the adrenal medulla release adrenaline, noradrenaline, and dopamine into the systemic circulation.[88] Circulating noradrenaline is therefore derived from both the adrenal medulla and diffusion into the circulation of noradrenaline secreted by postganglionic neurons at synapses in target tissues.[88]

Opioid receptors have been documented in the hypothalamus; for instance, the presence of μ opioid receptors in the hypothalamus has been confirmed by documenting hypothalamic binding of the highly specific μ opioid receptor agonist, D-ala^2-MePhe^4-Gly^5-ol-enkephalin (DAGO).[89] Opioid receptors have also been found in other brain regions involved in autonomic functions, including the medulla oblongata.[89; 90] Furthermore, light microscopic immunocytochemistry has shown that opioidergic and adrenergic neurons are co-localised in several distinct brain regions, including the hypothalamus.[91]

Injection of endogenous opioid peptides (e.g., endorphin) or exogenous opioids at different sites in the brain elicits cardiovascular and respiratory autonomic effects that vary depending on a number of factors such as species, dose, the specific brain region injected, route

of injection (i.e., intraventricular vs. injection into specific brain nuclei, systemic vs. central administration), receptor selectivity and affinity, ventilation (spontaneously vs. mechanically ventilated), and state of consciousness (i.e., anaesthetised vs. conscious).[9] For instance, injection of morphine, a μ opioid receptor agonist, into the POM of the anaesthetised rat caused an increase in f_H and SAP and a decrease in f_R and amplitude compared to baseline, whereas injection into the PVN of the anaesthetised rat, separated from the POM by only 700 μm, caused an increase in f_H and f_R but a decrease in SAP compared to baseline.[9; 92] Anaesthesia (e.g., with pentobarbitone), however, is a known confounder of opioid-induced cardiovascular effects.[9; 93] Indeed, injection under anaesthesia typically produces effects opposite of those produced by injection in conscious rats.[8] Central injections of μ opioid receptor agonists in conscious rats eliminates these confounding effects and might produce results that are more applicable to the white rhinoceros, which is usually injected with etorphine while conscious.[94; 95] In conscious rats, intracerebroventricular or intracisternal injection, or injection into the PVN or POM, of low doses of μ opioid receptor agonists (e.g., dermorphin, DAGO, morphine, β-endorphin) consistently increases sympathetic outflow.[80; 95-99] In a dose-response study, POM injection of DAGO was 10 times more potent with regard to elicitation of cardiovascular and respiratory effects than the δ opioid receptor agonist [D-Ala^2, D-Leu^5]-enkephalin (DADL), supporting the μ opioid receptor as the mediator of these effects in the POM.[93] This sympathetic outflow consists of an increase in f_H, which increases Qt, which then increases SAP (with a transient increase in SVR also contributing to the increase in SAP).[8] Core temperature increases as well.[96] Distinct changes in regional blood flow are also observed; blood flow to the skeletal muscle of the pelvic limb increases, whereas mesenteric blood flow decreases.[8; 96; 97] Increased plasma catecholamine concentrations are detected, and the catecholamine(s) released is dependent on the opioid

receptor agonist injected and its receptor selectivity.[80; 95; 96; 100; 101] For instance, intracisternal injection of β-endorphin increases concentrations of all three catecholamines, whereas morphine increases adrenaline concentration only, and DAGO increases concentrations of all three catecholamines significantly more than β-endorphin.[100] Transection of the rat spinal cord at C1 eliminates all responses to DAGO, suggesting that connection to the sympathetic neurons in the thoracolumbar spinal cord is necessary for sympathetic outflow. Injection of the κ opioid receptor agonist, dynorphin 1-13, into the POM and PVN of anaesthetised rats decreased f_H and f_R.[92]

In summary, although opioids clearly play a role in central autonomic control, their interaction with the autonomic nervous system is extremely complex and the subject of considerable controversy.[9] Given the many factors that influence the autonomic effects of opioids, one must exercise caution in extrapolating findings in rats to horses and white rhinoceros; however, the extensive scientific evidence demonstrating that ultra-potent opioids are capable of triggering massive sympathetic outflow in mammals supports the hypothesis that etorphine exerts some of its pathophysiological effects in the white rhinoceros in this way.

Glycolysis, Aerobic Metabolism, and Lactate

Normal Metabolism

An understanding of normal metabolism and lactate production is necessary to understand the significance of lactate concentration in the immobilised white rhinoceros. Complete oxidation of glucose for production of adenosine triphosphate (ATP) occurs in three stages: glycolysis (the Embden-Meyerhof pathway), the citric acid (Krebs or tricarboxylic acid [TCA]) cycle, and oxidative phosphorylation. These have been reviewed elsewhere, and the summary below is assembled from these sources.[102-104]

Glycolysis occurs in the cytoplasm of all cells. The *net* reaction per molecule of glucose is: glucose + 2 ATP + 2 NAD^+ → 2 $pyruvate^{-1}$ + 2 ATP + 2 $NADH_2$ + 2 H_2O + heat. (Note that 2 ATP are consumed and 4 ATP are produced per glucose molecule, resulting in a *net* gain of 2 ATP, as depicted on the right-hand side of the equation). The carrier molecules nicotinamide dinucleotide (NAD^+) and the related flavin adenine dinucleotide (FAD^+) can each bind 2 hydride ions (H^-) and two protons (H^+), or 2 hydrogen (H), to form $NADH_2$ or $FADH_2$ (NAD^+ + 2 H^- + 2 H^+ → NADH + H^+, or $NADH_2$). $Pyruvate^{-1}$ is an anion and conjugate base to pyruvic acid and is expressed as '$pyruvate^{-1}$' to emphasise this fact. Oxygen is not required in this stage.

The two $pyruvate^{-1}$ molecules diffuse across the outer mitochondrial membrane into the matrix, the site of the citric acid cycle, where each is converted irreversibly by $pyruvate^{-1}$ dehydrogenase into a molecule of acetyl-coenzyme A (CoA), generating a total of 2 CO_2 (which diffuse out of the mitochondria and are ultimately exhaled by the lungs) and 4 H, which are bound by NAD^+ to form 2 $NADH_2$. The two acetyl-CoA molecules that have now been formed from one glucose molecule then enter the citric acid cycle, where each combines with oxaloacetate, releasing the CoA portion and forming citrate. Citrate is then converted into a series of intermediates and ultimately back into oxaloacetate; these conversions produce more CO_2 and H, as well as ATP. For every 2 acetyl-CoA molecules that enter the cycle, 4 CO_2, 18 H (8 $NADH_2$ and 1 $FADH_2$), and 2 ATP are produced.

During the final stage, called oxidative phosphorylation, the majority of ATP formed from glucose metabolism is generated by oxidation of the H atoms released during the first two stages and carried by NAD^+ and FAD^+. The H is released, regenerating NAD^+ and FAD^+ , and split into H^+ and electrons (e^-); the e^- enter the electron transport chain where they are passed along a series of electron acceptors within the inner mitochondrial membrane, or cristae, of the

mitochondria. This e^- transport releases energy which is used to pump the H^+ across the inner membrane into the outer chamber of the mitochondria, creating a negative electrical potential in the inner chamber. The H^+ then flow back into the inner chamber down their electrical gradient; the energy derived from this H^+ flow is used by the enzyme ATP synthase to produce a further 34 ATP from adenosine diphosphate (ADP) and phosphate (PO_4^{-3}). The electrons eventually reach the acceptor called cytochrome A_3, or cytochrome oxidase, which uses 2 e^- to reduce elemental oxygen (O) to O^{-2} ($2\ e^- + 1/2O_2 \rightarrow O^{-2}$). The O^{-2} then combines with 2 H^+ to form H_2O. Thus, this stage is when the oxygen delivered to cells by the cardiopulmonary system is utilised. In all, 38 ATP have been produced.

In health, about 10% of pyruvate^{-1} produced by glycolysis does not diffuse across the mitochondrial membrane but is converted to lactate^{-1}, also an anion and the conjugate base of lactic acid (lactate^{-1} + H^+ ↔ lactic acid); thus, the terms lactate and lactic acid are not synonymous. At physiological pH, lactic acid is almost completely dissociated into lactate^{-1} and H^+. Most of the lactate^{-1} produced in health is generated in skeletal muscle and brain, although several other tissues contribute. Each pyruvate^{-1} molecule can be converted to one lactate^{-1} molecule. The conversion of a pyruvate^{-1} molecule to a lactate^{-1} molecule by the enzyme lactate dehydrogenase (LDH) consumes the 2 H from each $NADH_2$ (above) to regenerate 2 NAD^+; thus, lactate^{-1} production actually *consumes* H^+: 2 pyruvate^{-1} + 2 $NADH_2$ → 2 lactate^{-1} + 2 NAD^+. The small amount of lactate^{-1} ordinarily produced (~0.5 mmol L^{-1} in the horse) is transported to other tissues, such as the liver, renal cortex, and myocardium, where it can be converted back to glucose (gluconeogenesis) or converted to pyruvate^{-1}, which then enters the citric acid cycle.[105]

Metabolism During Hypoxia

Oxidative phosphorylation can continue normally until the oxygen partial pressure (PO_2) in the mitochondria reaches ~1-2 mm Hg; this critical PO_2 is known as the Pasteur point. Oxidative phosphorylation decreases when oxygen delivery (DO_2) to tissues is inadequate because oxygen is unavailable for reduction by cytochrome oxidase. Thus, H^+ accumulate. The law of mass action dictates that $NADH_2$ and $FADH_2$ are then unable to release H, and NAD^+ and FAD^+ cannot be regenerated (above). The end result is that pyruvate^{-1} and $NADH_2$/$FADH_2$ accumulate in the cytoplasm, which would halt glycolysis were it not for an alternative pathway that consumes pyruvate^{-1} and the H carried by $NADH_2$: the increased production of lactate^{-1}. Thus, lactate^{-1} production *regenerates* NAD^+, allowing glycolysis to continue to generate the small amount of ATP that can sustain the cell until oxygen becomes available once again. Thus, the metabolic acidosis that occurs when hyperlactatemia is present is caused not by lactate itself but by the concurrent accumulation of H^+ (although this concept is the subject of some controversy). Thus, hyperlactatemia can occur in the absence of metabolic acidosis. The accumulation of lactate and H^+ usually, however, occurs due to the same pathophysiological process (i.e., tissue hypoxia), which explains why hyperlactatemia and metabolic acidosis coexist frequently.

Hyperlactatemia is classified into Type A (inadequate oxygen supply) and Type B (adequate oxygen supply).[106] Type A can be further subdivided into relative and absolute hyperlactatemia. Relative hyperlactatemia is caused by an increase in VO_2 (e.g., from exercise, shivering, or tremors/seizures [observed in white rhinoceros during etorphine immobilisation]) whereas absolute hyperlactatemia is caused by a decrease in DO_2 (e.g., from decreased CaO_2 [observed in white rhinoceros] or Qt [*not* observed in white rhinoceros]). Type B is caused by

certain diseases, or drugs or toxins, including adrenaline and glucocorticoids. Both types likely play a role during white rhinoceros immobilisation (discussed further below).

Exercise

Exercise Physiology

The cardiovascular adjustments that occur in the human and non-human mammalian athlete during exercise have been well characterised and are reviewed in greater detail elsewhere.[107-110] These adjustments are mimicked by the physiological effects induced by etorphine in horses and white rhinoceros and might inform our understanding of etorphine's pathophysiological effects. Furthermore, certain pathological processes that occur in maximally exercising athletes could also shed light on how etorphine might cause hypoxaemia.

Muscles consume more ATP during exercise than at rest; the ATP must be regenerated by oxidative phosphorylation, as discussed above. An increase in oxidative phosphorylation consumes more oxygen and produces more carbon dioxide (CO_2), hence the rapid increase in VO_2 and VCO_2 at the onset of exercise; little to no lactate^{-1} production occurs (beyond basal production) because DO_2, the product of Qt and CaO_2, increases in response to the increase in VO_2 (below).[105; 111-116] The anaerobic threshold is defined as the greatest exercise intensity at which VO_2 accounts for a subject's entire energy requirement.[117] When the total work performed during exercise exceeds the athlete's capacity for aerobic metabolism, anaerobic metabolism commences. With light to moderate exercise, VO_2 and VCO_2 plateau within minutes; lactate^{-1} production increases but also plateaus.[113; 115; 118-121] Maximal oxygen consumption (VO_{2max}) is the VO_2 of an athlete during maximal exertion; the VO_{2max} of a horse can reach twice that of an elite human athlete (~160 mL minute^{-1} kg^{-1} vs. ~80 mL minute^{-1} kg^{-1}), despite the horse's lower basal metabolic rate.[122] The lactate threshold (T_{lac}), the VO_2 beyond which lactate concentration

begins to increase exponentially, is ~50% of VO_{2max} in both humans and horses.[119; 120] Beyond T_{lac}, a biphasic increase in VO_2 and VCO_2 occurs, with an initial rapid increase followed by a slower increase.[119]

The cardiovascular adjustments that occur during exercise are controlled by two general mechanisms working in concert, all designed to ensure that DO_2 increases in response to the increase in VO_2: 1) the 'central command', the descending signals from higher brain centres that activate somatomotor, cardiovascular, and respiratory systems in parallel at the onset of exercise or when exercise is anticipated and 2) reflexes, namely the 'exercise pressor reflex' and 'arterial baroreflex'.[123-125] Central command requires consciousness and thus would probably not play a role in the etorphine-anaesthetised white rhinoceros.

The 'exercise pressor reflex' is the constellation of cardiovascular changes produced by contracting skeletal muscles, which even occurs when muscle is stimulated to contract in anaesthetised animals.[108; 126-129] Mechano- and metaboreceptors (chemoreceptors) on type III (Aδ) and type IV (C) afferent neurons arising in skeletal muscle are stimulated by muscle contraction and skeletal muscle metabolites, respectively.[130] The specific metabolite that stimulates the type IV afferent neurons remains uncertain; candidate substances include $lactate^{-1}$, K^+, ATP, arachidonic acid, prostaglandins, and H^+.[127; 131] These afferent neurons conduct impulses into the spinal cord dorsal horn, where they synapse with neurons that ascend to the medulla oblongata.[130] The VLM, introduced earlier in this chapter and also called the 'pressor region', is a region of the medulla oblongata involved in regulation of the cardiovascular system during exercise.[132] The RVLM is excited during stimulation of types III and IV afferent neurons during muscle contraction and projects directly to the IML cell column of the thoracolumbar spinal cord, where it synapses with preganglionic sympathetic neurons.[84; 133] Stimulation of the

RVLM increases f_H and SAP.[84] Indeed, plasma catecholamine concentrations increase during exercise in both humans and horses, which is likely due to a combination of catecholamine release from the adrenal medulla and diffusion of noradrenaline out of synapses into the circulation.[112; 114; 134] Catecholamine concentrations in exercising horses increase to levels higher than those seen in humans and are directly related to exercise intensity.[112; 114] Adrenaline and noradrenaline act as agonists at α and β adrenoceptors. Increased f_H/tachycardia and contractility of the atria and ventricles (which increases SV) is primarily mediated by β_1 adrenoceptors in the heart.[88; 135; 136] The majority of the increase in DO_2 in exercising horses is caused by an increase in Qt, the product of f_H and SV, which can increase eight-fold between rest and maximal exertion.[111; 113] In the horse, tachycardia, which can peak at well over 200 beats minute^{-1}, is primarily responsible for the increase in Qt, while SV is unchanged.[105; 111; 113] Haemoglobin concentration is a major determinant of CaO_2; in the horse, splenic contraction, which is mediated by α_1 adrenoceptors, accounts for a significant increase in [Hb] (by 59-72% of its baseline value in one study) and can increase CaO_2 from 20 mL dL^{-1} to 30 mL dL^{-1} during galloping.[105; 111; 113; 137-139] Sympathetic outflow also causes changes in regional blood flow distribution. β_2 adrenoceptors on arterioles in the coronary circulation partially mediate 'exercise hyperaemia', the vasodilation that increases local blood flow to the heart during exercise.[88; 135; 140] However, α_1 receptors are present on the large resistance arteries of the coronary circulation and mediate vasoconstriction of these vessels. The significance of α adrenoceptors on smooth muscle in these large resistance arteries of the coronary circulation, as well as on smooth muscle of the arteriolar tree supplying skeletal muscles, will be elucidated below when the 'arterial baroreceptor reflex' is discussed. In inactive skeletal muscles and viscera, such as the kidneys, liver, and gastrointestinal tract, α_1 and α_2 adrenoceptors mediate vasoconstriction, to divert blood

away from these tissues, which then increase oxygen extraction to allow aerobic metabolism to continue.[141-145] Splanchnic arterial and venous vasoconstriction increase venous return to the right ventricle, which increases Qt.[146; 147]

Although neural mechanisms contribute to exercise hyperaemia, the majority of vasodilation in the coronary circulation and active skeletal muscles is caused by substances released from the muscles themselves (K^+, adenosine, ATP) or adjacent vascular endothelium (nitric oxide, prostaglandins); no one substance alone can evoke the massive increase in blood flow to these tissues during exercise, making it likely that multiple substances acting in concert are responsible.[148; 149] Local hypercapnia and hypoxaemia are also capable of causing vasodilation.[149-151]

The 'arterial baroreceptor reflex' plays a major role in the adjustment of SAP during exercise. Baroreceptors are free nerve endings located in the carotid arteries and aorta.[108] An increase in SAP causes a conformational change in the baroreceptor that increases action potential generation; the glossopharyngeal and vagus nerves transmit the action potentials to the NTS of the medulla.[108] The NTS then stimulates the nucleus ambiguus (NA) in the medulla to increase parasympathetic outflow, which decreases Qt and SVR.[108] Simultaneously, the NTS stimulates the caudal ventrolateral medulla (CVLM), which inhibits the RVLM via gamma (γ)-aminobutyric acid (GABA) transmission.[152; 153] A decrease in SAP decreases action potential generation and ultimately increases Qt and SVR. The baroreceptors respond to changes in SAP primarily by altering SVR rather than Qt during exercise.[154] The profound decrease in SVR caused by skeletal muscle vasodilation can outcompete the ability of the heart to generate a Qt sufficient to maintain adequate SAP, which could result in hypoperfusion of the brain and other tissues.[155] The arterial baroreceptor reflex maintains SVR to prevent systemic hypotension and

hypoperfusion.[108; 156] During exercise of increasing intensity, the baroreceptors 'reset' to maintain SAP at an increasingly greater value (below), which explains the increase in SAP during exercise.[105; 157] A kind of competition is thus created between vasodilation (to increase the delivery of oxygen and other nutrients) and vasoconstriction (to ensure preservation of SAP). To deal with these competing needs, α_1 adrenoceptors on the feed arteries and proximal arterioles mediate vasoconstriction, providing enough resistance to maintain SAP and limiting skeletal muscle blood flow to some extent, while the distal, smallest arterioles dilate to ensure the muscle receives adequate blood flow.[158; 159] The observation that vasocontriction in response to sympathetic stimulation (e.g., sympathetic nerve stimulation or intra-arterial noradrenaline infusion) is significantly attenuated in exercising muscle in direct proportion to intensity of exercise was termed 'functional sympatholysis'.[158] Vasoconstriction mediated by α_2 adrenoceptors appears to be more susceptible to attenuation than that mediated by α_1 adrenoceptors; this finding is consistent with the observation that more α_2 than α_1 adrenoceptors are expressed on smooth muscle of distal arterioles.[159; 160] The exact mechanism behind 'functional sympatholysis' is unclear, however, some local mechanism appears to be involved, as mentioned above.[161]

Because Qt cannot increase in proportion to VO_2, tissues must meet their oxygen needs by extracting more oxygen from the blood.[111; 113] The increased oxygen extraction decreases $P\bar{v}O_2$, $S\bar{v}O_2$, and $C\bar{v}O_2$, increases the arteriovenous oxygen content difference ($CaO_2 - C\bar{v}O_2$), and increases the oxygen extraction ratio (OER), which is normally 0.2-0.3, to as high as 0.8 during exercise.[105; 111; 113; 137] $C\bar{v}O_2$ serves as an important oxygen reserve, and decreased $C\bar{v}O_2$ exacerbates hypoxaemia. $C\bar{v}O_2$ would decrease progressively and be exhausted quickly if the respiratory system was unable to adapt by increasing V_A, which increases linearly with VO_2 in

horses, to raise PAO_2.[105; 115] Indeed, in ponies during near-maximal to maximal exertion (galloping) and in horses during prolonged submaximal exertion (i.e., 60% of VO_{2max}), ventilation increases and $PaCO_2$ decreases due to an increase in both f_R and tidal volume (V_T), while PaO_2 remains the same or increases.[105; 137] This effect is consistent with the finding of Haw et al. (2014) that $PaCO_2$ in field-immobilised white rhinoceros, which run in response to helicopter pursuit and darting, was lower than that of white rhinoceros immobilised in bomas, but PaO_2 was similar.[6] In Thoroughbred racehorses, it is not until near-maximal to maximal exertion that $PaCO_2$ begins to increase; this hypercapnia indicates that the evolution of the capacity of the horse's cardiovascular system to support high-intensity exercise has outstripped its respiratory system's ability to increase ventilation.[105; 162] The mechanism(s) by which ventilation increases to match VO_2 have not been elucidated fully. An increase in V_A is impossible in the white rhinoceros once catatonia and recumbency have occurred because of the profound respiratory depression caused by etorphine.

Exercise-Induced Arterial Hypoxaemia (EIAH) and Exercise-Induced Pulmonary Haemorrhage (EIPH)

Hypoxaemia with an increased $PA\text{-}aO_2$ during exercise is a well-documented phenomenon in healthy, fit, human and equine athletes.[116; 163; 164] The $PA\text{-}aO_2$ increases progressively with exercise intensity and VO_2.[116; 165; 166] The pathophysiology of this hypoxaemia appears to be multifactorial. Wagner et al. (1989) found, using the multiple inert gas elimination technique in maximally exercising horses, that gas diffusion limitation was primarily responsible, with a smaller contribution from a mild ventilation:perfusion (V:Q) mismatching (although V:Q mismatching can be severe in some horses).[116] In humans, V:Q mismatching contributes proportionally more to EIAH than it does in horses.[165-167]

The normal pressures in the cardiac chambers and great vessels of resting humans and horses have been published.[105; 166; 168-170] During maximal exertion in both species, pulmonary hypertension occurs.[166; 168; 171] The mPAP of a resting, healthy horse is ~30 mm Hg (relative to atmospheric pressure), whereas at VO_{2max}, mPAP can more than double, with reports of mPAP > 100 mm Hg.[168; 170] This pulmonary hypertension appears to be due to the dramatic increase in Qt from that at rest, as described above.[105; 113; 172] Such an increase could significantly decrease erythrocyte transit time through the pulmonary vascular bed, limiting the time for equilibration of oxygen between alveolus and erythrocyte and producing gas diffusion limitation.[173] Furthermore, an increase in PAOP from that at rest occurs during exercise in both species, and in people, the increase is greater is untrained subjects.[170; 174] Pulmonary arterial occlusion pressure increased in one equine study from ~18 mm Hg at rest to ~56 mm Hg.[170] This increase may be due to incomplete ventricular relaxation and increase in end-diastolic left ventricular pressure (LVEDP).[174]

The discovery of pulmonary hypertension during exercise generated further investigations to determine if Pc was also increased from that at rest.[168; 172] Although Pc cannot be measured directly using currently available technology, it has been estimated in horses using the arterial occlusion technique, which demonstrated that Pc more than doubled between rest (~20 mm Hg) and VO_{2max} (> 40 mm Hg).[168]

Understanding how changes in pressure and flow in the pulmonary vascular bed relate to EIAH requires knowledge of Starling's forces, first described in 1896 and later revised.[175-177] The rate of filtration of fluid (J_v in volume per unit time) across a semipermeable membrane = K_f ([Pc – Pis] – σ[Πp – Πg]) where K_f is the filtration coefficient constant, the product of hydraulic conductivity and filtration surface area (a measure of the permeability of the endothelial barrier

to water movement); Pc and Pis are the hydrostatic pressures in the pulmonary capillary lumen and the pericapillary interstitial space, respectively; Πp and Πg are the protein (colloid) osmotic (oncotic) pressures of the plasma and the subglycocalyx fluid, respectively; and σ is the Staverman reflection coefficient.[176; 177] The sum of the forces causing filtration of fluid out of the capillary normally slightly exceeds the sum of forces causing absorption of fluid into the capillary, resulting in a slightly positive J_v.[175; 176] If Pc were to increase, J_v would increase, increasing filtration of fluid out of the pulmonary capillaries.[175-178] If the rate of filtrate formation exceeded the rate of removal by pulmonary lymphatics, pulmonary oedema would ensue.[175-177] While lymphatics can increase their rate of absorption of filtrate several-fold, a critical J_v exists beyond which no further accommodation by the lymphatics occurs, and interstitial oedema ensues.

Pulmonary oedema can occur in human endurance atheletes, swimmers, and triathletes. Radiography and ultrasonography have documented signs of oedema, and reports of athletes with clinical signs of pulmonary oedema, including hypoxaemia, crackles on pulmonary auscultation, dyspnea, and sanguineous, frothy sputum have been published.[179-181] In one study, 65% of men running at maximum capacity for 7 minutes had radiographic evidence of pulmonary oedema.[179] Although early observations in horses appeared to confirm an increase in extravascular lung water during maximal exertion, Wilkins et al. (2001) were not able to document an increase in extravascular lung water using the dual indicator-dilution technique.[171] However, mPAP only increased to ~75 mm Hg in that study and may have been insufficient to cause stress failure.[171] Vengust and colleagues (2006) demonstrated large increases in transpulmonary fluid fluxes in race-fit Standardbreds during exercise.[178]

If Pc continues to increase, J_v will also increase; eventually, the interstitium will no longer be able to accommodate the excess filtrate, and fluid will flood the alveoli, causing alveolar oedema. Oedema could explain the reports of white froth at the nares of rhinoceros that have died during immobilisation (Prof. Leith Meyer, personal communication). Exercise-induced pulmonary haemorrhage (EIPH) is a common disorder in equine athletes and is characterised by the expulsion of sanguineous fluid or foam, or frank blood (epistaxis), from the nares during maximal exertion.[182] It appears to be due to very high (75-100 mm Hg) pulmonary capillary transmural pressure (Ptm, the pressure difference between the capillary lumen and alveolus), which leads to stress failure of pulmonary capillaries and subsequent haemorrhage into the alveoli.[183; 184] Sanguineous foam has also been reported at the nares of rhinoceros that have died (Prof. Leith Meyer, personal communication). Multiple ultrastructural changes in the lungs of horses known to have EIPH have been described.[184] Although very high pressure is required to cause EIPH in the horse, the Ptm required to cause stress failure of pulmonary capillaries is species-specific (e.g., ~37 mm Hg in rabbits compared to ~66 mm Hg in dogs), and this value is unknown in the white rhinoceros.[185] Alveolar oedema and haemorrhage would cause venous admixture due to gas diffusion limitation and V:Q mismatching, producing hypoxaemia. Given that the cardiopulmonary changes induced by etorphine are similar to those induced by maximal exercise, it seems plausible that oedema, and possibly even stress failure of pulmonary capillaries in some rhinoceros, could play a role in etorphine-induced hypoxaemia.

Scope of the Thesis

Problem Statement

Chemical immobilisation of the white rhinoceros with etorphine is necessary for its conservation but causes severe physiological derangements, including hypoxaemia, which have

resulted in mortalities.[1] Currently, the mechanisms by which etorphine causes these derangements are incompletely understood, and many questions remain despite an excellent body of recently published research. Hypoxaemia can be caused by decreased inspired oxygen fraction (FiO_2, not applicable in this case), hypoventilation, diffusion limitation, V:Q mismatching, and shunting, and can be exacerbated by decreased mixed venous oxygen content ($C\bar{v}O_2$). Although hypoventilation is a well-known physiological derangement in the etorphine-immobilised white rhinoceros, an increased $P_A\text{-}aO_2$ has been documented, demonstrating that hypoventilation, which does not increase $P_A\text{-}aO_2$, cannot be the sole mechanism behind the hypoxaemia. Is diffusion limitation, V:Q mismatching, or shunting responsible, or do all three play a role to some extent? What might cause each of these in the etorphine-immobilised white rhinoceros? What is the $C\bar{v}O_2$ and might a mixed venous hypoxaemia secondary to the hypermetabolism documented in previous research also play a role? Are the other observed physiological derangements, such as acidaemia, tachycardia, systemic arterial hypertension, and tremors related to the hypoxaemia, and if so, how? Can one pathophysiological mechanism, such as sympathetic outflow, which is supported by data from other studies, explain all these problems? A comprehensive understanding of these mechanisms will facilitate the development of rational preventative or therapeutic measures to improve the safety of white rhinoceros immobilisation. Elucidation of these mechanisms, in turn, requires measurement of pulmonary pressures and Qt, as well as SAP, arterial and mixed venous blood gases and acid-base status, V_E, and metabolic rate. Measurement of pulmonary pressures, Qt, and mixed venous blood gas partial pressures and acid-base status requires catheterisation of the pulmonary artery, a challenging task given the rhinoceros' thick skin and size. Thus, my colleagues and I developed and refined a technique for pulmonary arterial catheteristion and thermodilution Qt

measurement. These techniques will enable measurement of outcome variables that give useful information about etorphine and also facilitate objective assessment of candidate strategies for treating its adverse effects.

Aims

The aims of the preliminary study described in Chapter 2 were to 1) develop and refine a techique for pulmonary arterial catheterisation in the white rhinoceros, specifically to design and test a custom-built, balloon-tipped (Swan-Ganz type) pulmonary arterial catheter (PAC) suitable for insertion in the white rhinoceros, and 2) determine the effects of a bolus of supplemental etorphine administered IV to white rhinoceros immobilised with etorphine-azaperone IM, followed by butorphanol IV, on PAP, Qt, f_H, SAP, and blood gases using the PAC.

Chapters 3 and 4 describe a study using boma-habituated white rhinoceros to eliminate the confounding effects of psychological stress and exertion in the field. My aims in Chapter 3 were to 1) elucidate the mechanisms contributing to the hypoxaemia observed in etorphine-immobilised white rhinoceros and 2) determine if etorphine induces sympathetic upregulation. In Chapter 4, my primary aim was to determine if butorphanol administered IV to etorphine-immobilised white rhinoceros increased DO_2 more than saline. To these ends, I designed a crossover study in which each rhinoceros was immobilised with etorphine only, followed by saline (treatment ES) or butorphanol (treatment EB). After positioning in lateral recumbency (t = 0), PAP, PAOP, SAP, Qt (by thermodilution), arterial and mixed venous blood gases and acid-base status, VO_2 and VCO_2, and plasma nordrenaline concentrations were measured at t = 30, saline or butorphanol were administered at t = 37, and the above variables were measured again at t = 40 and 50. I achieved my aims by examining etorphine's effects over time in ES (Chapter

3) and by comparing values for the above variables in ES and EB at each sampling point (Chapter 4).

Hypotheses

We conducted a preliminary study, described in Chapter 2, in which the effects of a supplemental, IV bolus of etorphine on some cardiopulmonary variables were determined in boma-habituated white rhinoceros already immobilised with etorphine and azaperone, followed by butorphanol IV. For this preliminary study, I hypothesised that a supplemental IV etorphine bolus would upregulate sympathetic outflow and produce an increase in mPAP and Qt in the white rhinoceros.

With the results of the preliminary study in mind, I developed hypotheses for the studies described in Chapters 3 and 4. For the etorphine study (Chapter 3), I hypothesised that 1) mechanisms other than opioid-induced hypoventilation contribute to the hypoxaemia observed in etorphine-immobilised white rhinoceros, and 2) etorphine induces sympathetic upregulation that decreases over the period of immobilisation. For the butorphanol study (Chapter 4), I hypothesised that butorphanol given to white rhinoceros immobilised with etorphine increases DO_2 to tissues.

CHAPTER 2 THE EFFECTS OF A SUPPLEMENTAL ETORPHINE DOSE ON PULMONARY ARTERY PRESSURE AND CARDIAC OUTPUT IN IMMOBILISED, BOMA-HABITUATED WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*): A PRELIMINARY STUDY

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Abstract

The effects of etorphine on the pulmonary vascular system of white rhinoceros (*Ceratotherium simum*) have not been described and could play a role in the severe hypoxaemia that develops after immobilisation with etorphine-based drug combinations. Characterisation of these effects requires measurement of pulmonary vascular pressures and cardiac output (Qt). To refine a technique for pulmonary arterial catheterisation, five boma-habituated white rhinoceros (three females and two males weighing 1,012-1,572 kg) were immobilised by remote injection with etorphine plus azaperone, followed by butorphanol. This afforded the opportunity to perform a pilot study and acquire preliminary measurements of pulmonary arterial pressure (PAP) and Qt before and after supplemental etorphine given intravenously (IV). Ultrasonographic guidance was used to insert a sheath introducer into a linguofacial branch of a jugular vein. A 160 cm long pulmonary arterial catheter (PAC) with a balloon and thermistor was then passed through the introducer and positioned with its tip in the pulmonary artery. It was not long enough to permit wedging for measurement of pulmonary arterial occlusion pressure (PAOP). Mean PAP (mPAP) was 35 (32, 47) mm Hg [median (minimum, maximum)] and increased (P = 0.031) by 83 (28, 106)% after supplemental etorphine. Thermodilution Qt was 120 (92, 145) L minute^{-1} and increased 27 (3, 43)% (P = 0.031). Heart rate (f_H) was 100 (88, 112) beats minute^{-1} and increased 20 (4, 45)% (P = 0.031), whereas arterial partial pressure of oxygen (PaO_2) was 35 (30, 94) mm Hg and decreased 47 (20, 72)% (P = 0.031). The cardiovascular observations could result from etorphine-induced generalised sympathetic outflow, as has been reported in horses. Further studies of etorphine in isolation are needed to test this suggestion and to discern how the changes in pulmonary vascular pressures and blood flow might relate to hypoxaemia in etorphine-immobilised white rhinoceros.

Keywords: Cardiac output, *Ceratotherium simum*, etorphine, pulmonary arterial catheter, pulmonary artery pressure, white rhinoceros.

Introduction

Life threatening hypoxaemia, hypercapnia, acidaemia, and other serious physiological derangements are ubiquitous during chemical capture of wild white rhinoceros (*Ceratotherium simum*).[5; 7; 59-61] Chemical immobilisation of this species in the field is usually carried out by aerial darting with the potent μ opioid receptor agonist, etorphine, co-administered with a tranquiliser (e.g., azaperone) and subsequent partial reversal with a μ opioid receptor antagonist/κ opioid receptor agonist (e.g., butorphanol).[5; 7] Extreme exertion during darting might contribute to the hypoxaemia and other physiological perturbations. However, white rhinoceros exhibit severe hypoxaemia, hypercapnia, respiratory acidaemia, tachycardia, and systemic hypertension after darting with this combination of drugs in a boma where exertion is minimal.[2-4]

The above observation suggests that one or more of the drugs used in capture are primarily responsible for the hypoxaemia and other perturbations. When given alone to goats, etorphine causes hypoxaemia, pulmonary hypertension, hypoventilation, and hypercapnia.[76] The latter observations suggest that etorphine might be the cause of the various perturbations observed during capture of white rhinoceros. The pulmonary vascular effects of etorphine in white rhinoceros have yet to be investigated either in combination with other drugs, as described above, or in isolation.

In-depth systematic evaluation of the pulmonary effects of capture in general, and etorphine in particular, in white rhinoceros will require measurement of pulmonary vascular pressures and cardiac output (Qt). In turn, these measurements will require catheterisation of the

pulmonary artery. Conventionally, in smaller species, a pulmonary arterial catheter (PAC) is introduced percutaneously *via* a jugular vein. Preliminary investigations using ultrasound imaging suggested that tissue depth over the jugular vein renders this percutaneous route impractical even in young white rhinoceros; however, those investigations suggested the linguofacial vein as a likely alternative access route.

In order to refine a technique for pulmonary arterial catheterisation, my colleagues and I had the opportunity to perform a pilot study on five boma-habituated white rhinoceros. This allowed them to assess a pulmonary arterial catheter design and to attempt to measure pulmonary artery pressure (PAP) and Qt for the first time in rhinoceros. The white rhinoceros were immobilised with etorphine plus azaperone, followed by butorphanol. Once instrumentation was complete and a set of measurements had been collected, a supplemental dose of etorphine was given intravenously (IV). This supplementation mimics the conditions that might pertain if an increment of etorphine were to be given in order to prolong immobilisation in the field, and it allowed changes in PAP and Qt after supplemental etorphine to be observed. In the horse, which is closely related phylogenetically to rhinoceros, sympathetic tone increases after etorphine administration hence PAP and Qt were expected to increase with the supplemental etorphine in the rhinoceros.[1,15]

Materials and Methods

This study was approved by the University of Pretoria (UP) Animal Ethics Committee (AEC) (project number V101-15) and the South African National Parks (SANParks) Animal Use and Care Committee (AUCC, reference number 001/16).

Five white rhinoceros (one adult female, two sub-adult females, one sub-adult male, and one male calf), weighing between 1,012 kg and 1,514 kg, were captured in Kruger National Park

(23°49´60 S, 31°30´90 E; altitude: 317 m above sea level), South Africa, and habituated to captivity over at least 6 months. The rhinoceros were housed individually in rhinoceros-specific holding pens (bomas) and were given *ad libitum* water and a mixture of lucerne (*Medicago sativa*) and tef (*Eragrostis tef*) hay. Faeces were removed from the enclosures, water troughs cleaned, and food replaced daily. The health of the rhinoceros was evaluated daily by trained personnel using a scoring system based on feed intake; volume, consistency, and colour of faeces; and behaviour.[186]

Rhinoceros were darted in the holding pens with a combination of etorphine (Elanco, Kempton Park 1619, South Africa; 9.8 mg ml^{-1}) and azaperone (Janssen Pharmaceuticals, Halfway House 1685, South Africa; 40 mg ml^{-1}). Drugs were administered intramuscularly (IM) in the nuchal hump or rump using a 3.0 mL plastic dart with a 60 mm uncollared needle fired from a compressed air rifle (DAN-INJECT International S.A., Skukuza, South Africa). Doses were selected based on previously measured body mass and were 1.98 (1.86, 2.26) [median (minimum, maximum)] µg kg^{-1} of etorphine and 0.019 (0.015, 0.026) mg kg^{-1} of azaperone. Once a rhinoceros could be safely approached, it was blindfolded and positioned in either right (n = 3) or left (n = 2) lateral recumbency. Butorphanol (5 mg for every 1 mg etorphine, or 0.01 mg kg^{-1}, Wildlife Pharmaceuticals Pty Ltd, White River, South Africa) was administered IV as soon as the animal was positioned in lateral recumbency.

After aseptic skin preparation, a 22 standard wire gauge (SWG), 2.5 cm over-the-needle IV catheter (Nipro Medical Corporation, Bridgewater, New Jersey, USA) was inserted into a medial auricular artery. Mean systemic arterial pressure (mSAP) was measured using a transducer (TranStar 60-inch Single Monitoring Kit, Ref MX950T, Smiths Medical ASD, Inc., Dublin, Ohio, USA) connected to a physiological monitor (Cardiocap/5, Datex-Ohmeda, GE

Healthcare, Helsinki 00510, Finland). When a transducer is plugged into this monitor, the monitor automatically performs a static calibration at 100 mm Hg. The transducer was zeroed to atmospheric pressure at the level of the *manubrium sterni* before the beginning of data collection for each rhinoceros and then again just before administering supplemental etorphine. Both transducer and monitor give results that are accurate (within ± 2 mm Hg) between 0 and 300 mm Hg (technical reference manuals for the above transducer and monitor); this was confirmed *post hoc* for the range of pressures measured using a mercury manometer. Arterial blood was also sampled anaerobically from the catheter for immediate analysis of blood gas tensions using the VetScan i-STAT 1 Handheld Analyser and pre-calibrated CG8+ cartridges (Abbott Point of Care Inc., Princeton, New Jersey, USA). Values for blood gas tensions were corrected to pulmonary artery temperature. Heart rate (f_H) was determined by auscultation of the thorax. The linguofacial branch of the jugular vein was located just caudal to the angle of the mandible (Figure 2-1) using ultrasonography (Hitachi Noblus ultrasound system fitted with a C25 convex 5 – 1 MHz transducer, Hitachi Aloka Medical, Wallingford, Connecticut, USA). Following standard aseptic skin preparation, a keyhole incision was made through the skin overlying the vein. Using ultrasonographic guidance, a 6-8 cm long, 12 g needle (Intraflon 2, Vygon, 95440-Ecouen, France) was inserted through the incision and into the vein. Once venous blood was observed in he needle hub, a wire guide was inserted through it. With the wire guide *in situ*, the needle was removed, and a dilator and 11 Fr (3.67 mm) percutaneous sheath introducer (Super Arrow-Flex Sheath Set, Teleflex, Morrisville, North Carolina, USA) were inserted over the wire guide into the vein. After removal of the dilator, a sterile, custom-built, 160 cm, 7 Fr (2.33 mm) Swan-Ganz-type thermodilution PAC (Gaeltec Devices, Dunvegan, Isle of Skye, Scotland IV55 8GU) was inserted through the introducer and down the jugular vein.

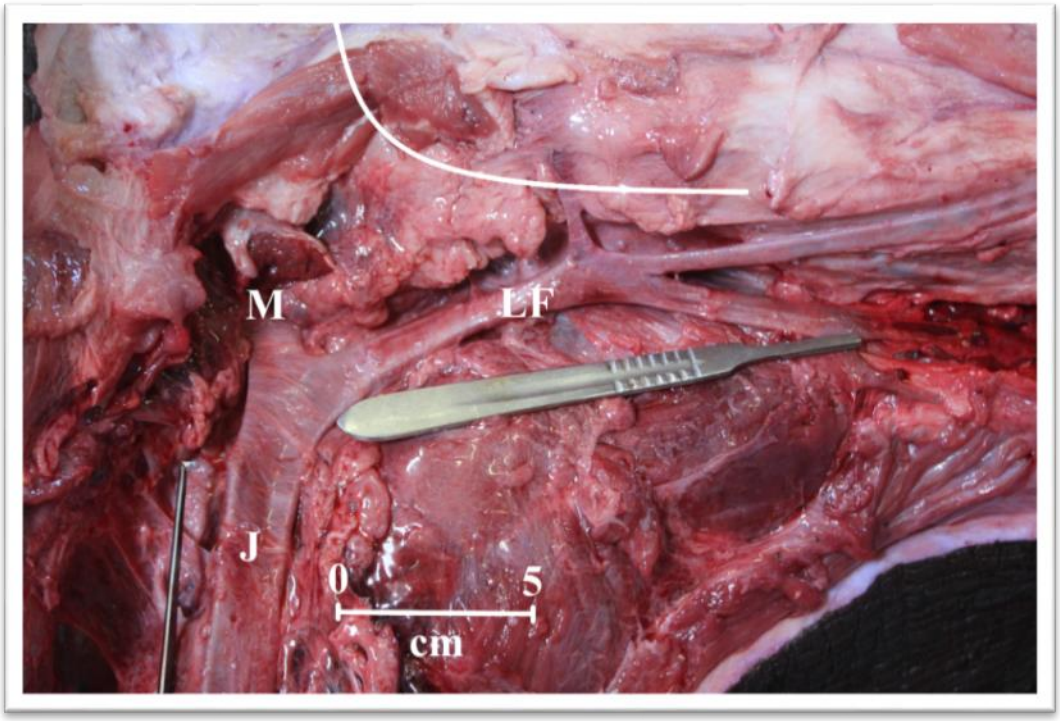


Figure 2-1 Dissection of the right linguofacial vein, ventrolateral view, in a white rhinoceros calf killed by poachers. Overlying skin, subcutis, muscle, and glandular tissue have been removed. Rostrad is to the right. The curved white line represents the angle of the mandible. J, jugular vein; M, maxillary vein; LF, linguofacial vein.

Pressure in the catheter was measured using a transducer (TranStar 60-inch Single Monitoring Kit, Ref MX950T) connected to another physiological monitor (Advisor Vital Signs Monitor, Smiths Medical, Dublin, Ohio, USA). As with the monitor used to measure SAP, when a transducer is plugged into this monitor, it also automatically performs a static calibration at 100 mm Hg. The transducer was zeroed to atmospheric pressure at the level of the *manubrium sterni* before the beginning of data collection for each rhinoceros and then again just before administering supplemental etorphine. This transducer and physiological monitor also give results that are accurate (within ± 2 mm Hg) between 0 and 300 mm Hg (technical reference

manuals for the above transducer and monitor). This was also confirmed *post hoc* for the range of pressures measured using a mercury manometer. The tip of the catheter was passed through the right heart and into the pulmonary artery while observing the characteristic pressure waveforms on the monitor. The ability of the catheter to wedge in a branch of the pulmonary artery was assessed by inflating and deflating the balloon on the catheter while observing the pressure waveform.

Cardiac output was measured by thermodilution (PM-9000 Vet Veterinary Portable Multi-Parameter Patient Monitor, ShenZhen Mindray Bio-Medical Electronics Co., Nanshan, ShenZhen, China). The thermistor in the PAC had the same properties as the thermistor used in ARROW balloon thermodilution catheters (Teleflex, Morrisville, North Carolina, USA). The appropriate computation constant for that thermistor (0.308) and an injectate volume of 6 mL were entered into the multiparameter patient monitor. Sixty mL of ice-cold 0.9% saline was injected by hand as rapidly as possible (< 3 seconds) through the side port of the introducer in the linguofacial vein. The same individual gave all injections of indicator (RG). After multiplying by a factor of 10, the median of 3 to 5 sequential measurements was calculated and recorded; no values were discarded. Supplemental etorphine 0.30 (0.23, 0.36) µg kg^{-1} was given IV 61 (53, 76) minutes after the initial injection of etorphine and azaperone. Pulmonary and systemic pressures, Qt, f_H, and arterial blood gases were measured within the 20 minutes before supplemental etorphine was given IV and again within the 10 minutes after it was given.

The rhinoceros were prepared for recovery after the last sampling point by removing all monitoring devices, removing the catheters and linguofacial vein introducer, applying pressure until adequate haemostasis had been achieved, administering an antibiotic (florfenicol, Nuflor, ~20 mg kg^{-1} IM, Merck & Co./Merck Sharp & Dohme Corp., Halfway House, South Africa) and

non-steroidal anti-inflammatory drug (meloxicam, Metacam, ~0.1 mg kg^{-1} IV, Boehringer Ingelheim, Randburg, South Africa), and clearing the boma of people. At this time, 0.11 (0.09, 0.14) mg kg^{-1} naltrexone (Kyron Laboratories, Benrose, South Africa) was administered IV to antagonise the etorphine.

A priori, mean PAP (mPAP) and Qt were selected as the variables of primary interest. Commercial software was used for data analysis (GraphPad Prism 7 for Windows, version 7.03, 2017, GraphPad Software, Inc., La Jolla, California, USA). Because of the small sample size, values were assumed to be non-normally distributed, hence the nonparametric Wilcoxon signed-rank test, one-tailed, was used to test the significance of differences between pre- and post-etorphine values. Differences between pre- and post-etorphine f_H, mSAP, arterial partial pressure of oxygen (PaO_2), and arterial partial pressure of carbon dioxide ($PaCO_2$) were also tested using the Wilcoxon signed rank test. Data are presented as median (minimum, maximum).

Results

Time to lateral recumbency after darting was 9 (8, 11) minutes. The pulmonary artery was accessed in all five rhinoceros with this catheter. However, the catheter could not be wedged in the pulmonary arterial bed of any of them. Pulmonary artery temperature of the rhinoceros prior to supplemental etorphine was 37.4 (35.8, 37.8)°C, and 37.7 (36.2, 38.2)°C after supplemental etorphine. Ambient temperature was 22.4 (17.7, 28.1)°C at the start of data collection.

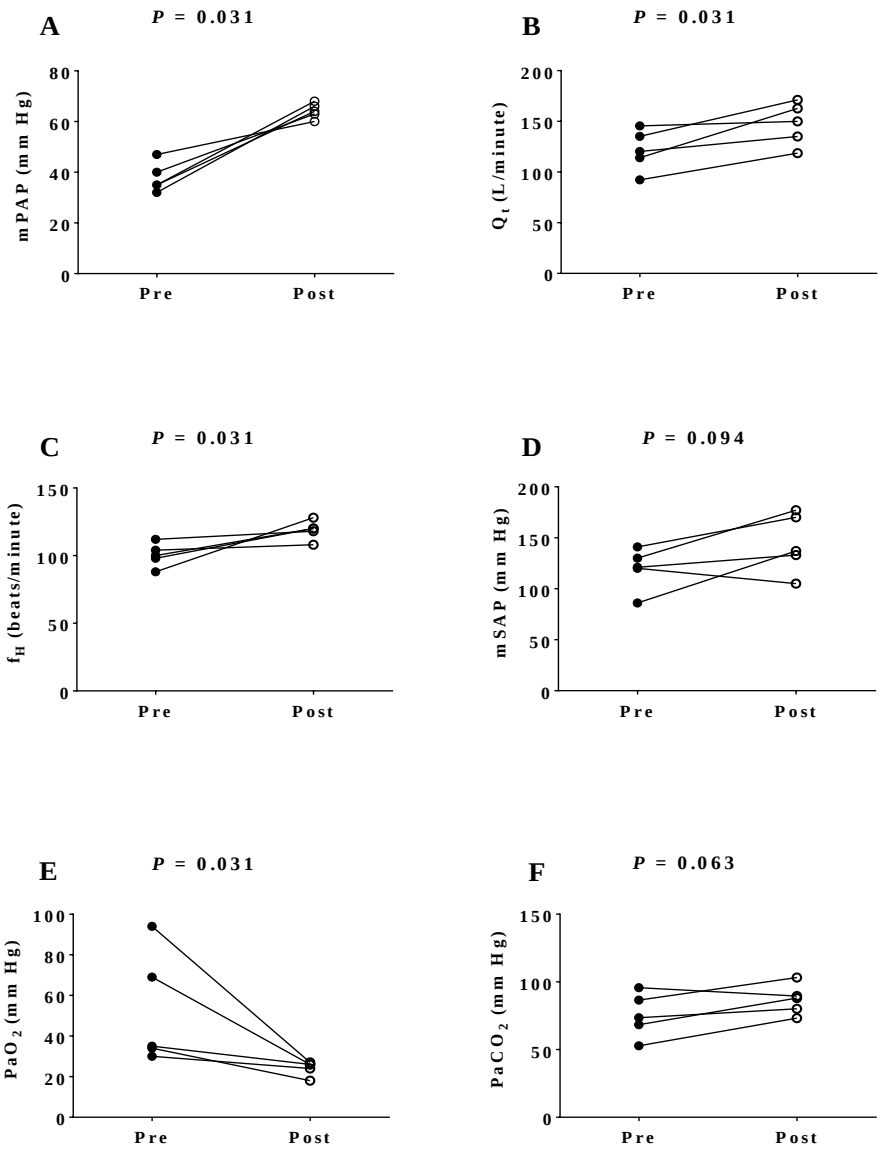


Figure 2-2 A) mean pulmonary arterial pressure (mPAP), B) cardiac output (Qt), C) heart rate (f_H), D) mean systemic arterial pressure (mSAP), E) arterial oxygen partial pressure (PaO_2), and F) arterial carbon dioxide partial pressure ($PaCO_2$) in five white rhinoceros before and after supplemental etorphine, 0.25-0.5 mg intravenously (IV). 'Pre' indicates measurements (closed circles) taken before supplemental etorphine; 'post' refers to those taken after supplemental

etorphine (open circles). The Wilcoxon signed-rank test, one-tailed, was used to test the significance of differences between pre- and post-etorphine values.

Mean pulmonary artery pressure was 35 (32, 47) mm Hg and increased 83 (28, 106)% (P = 0.031) to 64 (60, 68) mm Hg after the bolus of etorphine (Figure 2-2A). Cardiac output was 120 (92, 145) L minute^{-1} and increased 27 (3, 43)% (P = 0.031) to 150 (119, 171) L minute^{-1} after the bolus of etorphine (Figure 2-2B). Heart rate increased by 20 (4, 45)% (P = 0.031, Figure 2-2C) while mSAP increased in four rhinoceros and decreased in one (P = 0.09, Figure 2-2D). Arterial oxygen partial pressure decreased by 47 (20, 72)% (P = 0.031, Figure 2-2E), whereas $PaCO_2$ increased in four rhinoceros and decreased in one (P = 0.06, Figure 2-2F).

Time from darting to standing after immobilisation was 79 (67, 92) minutes. All rhinoceros were immobilised and recovered uneventfully.

Discussion

These experiments confirm that pulmonary arterial catheterisation and thermodilution Qt measurement are feasible in anaesthetised white rhinoceros. They also show that in rhinoceros of this size, the PAC needs to be longer than 160 cm in order to wedge in a branch of the pulmonary artery if the linguofacial vein is used for vascular access. Wedging is necessary to measure pulmonary arterial occlusion pressure (PAOP).

Mean pulmonary artery pressure was ~35 mm Hg before the supplemental dose of etorphine was given. Normal values for PAP are not available for white rhinoceros; however, these values are similar to those reported in standing horses.[168; 187; 188] The location of the tip of the PAC might have been several centimeters above or below the reference level (*manubrium sterni*) leading to an unmeasured hydrostatic offset to the values for mPAP. One of the authors has described previously a method for correcting for this but, unfortunately, the method requires

continuous pressure recording which was not available for these experiments.[168] Nevertheless, the pulmonary arterial catheter tip was not manipulated during pressure observations hence the *absolute changes* (although not percentage changes) in pressure observed after etorphine are immune from this offset.

Following a relatively small, supplemental dose of etorphine, mPAP increased ~83% and Qt increased ~27%. The increase in mPAP with etorphine is qualitatively consistent with observations in goats and would constitute severe pulmonary hypertension (defined as mPAP > 30-45 mm Hg, depending on the study) in a person.[189] Cardiac output decreased after etorphine in goats rather than increased as it did in the white rhinoceros.[76] Normal values for Qt are not available for white rhinoceros. However, applying allometric scaling principles to the body masses of these animals predicts that their normal Qt would be 55 (51, 70) L minute^{-1}.[122] Allometric scaling predicts that normal f_H in these animals would be 42 (39, 43) beats minute^{-1}; this is similar to f_H measured in unmedicated, standing white rhinoceros.[44; 122] Before supplemental etorphine, the values for both Qt and f_H that were measured were more than two times higher than their allometric correlates. The increase in Qt, f_H, and apparent increase in SAP that was observed after supplemental etorphine in rhinoceros agrees qualitatively with those caused by etorphine in horses; however to my knowledge, there are no data available for PAP in horses after etorphine.[67; 68; 70] In the horse, it has been postulated that the increase in Qt, f_H, and SAP are the consequence of upregulated sympathetic nervous activity with etorphine; this is supported by studies in horses that demonstrate increased plasma noradrenaline concentrations after etorphine administration, and mitigation of tachycardia and systemic arterial hypertension by preemptive β adrenoceptor blockade.[67; 75] In white rhinoceros immobilised with etorphine and azaperone, plasma adrenaline concentration correlates positively and negatively, respectively,

with tremor intensity and PaO_2, lending indirect support to a thesis that sympathetic upregulation might be responsible for the increases in PAP, Qt, and f_H reported here after etorphine.[7] Most of the rhinoceros were hypoxaemic and all were hypercapnic before supplemental etorphine, and the supplemental etorphine appeared to exacerbate the hypoxaemia and hypercapnia. Because hypoxaemia and hypercapnia likely upregulate sympathetic activity, these data do not allow distinction between a possible direct effect of etorphine on sympathetic tone and an indirect effect mediated by inhibition of pulmonary function.

All Stewart-Hamilton indicator dilution methods for Qt, such as the thermodilution method used here, assume that the indicator is conserved. The transit time of the indicator—ice-cold saline—between the injection site in the linguofacial vein and the thermistor in the pulmonary artery was longer than it would have been had the injection been made into the right atrium or the jugular vein, as is usual in smaller species; this almost certainly caused loss of thermal indicator (by cooling of perivascular tissue) and, thus, systematic overestimation of true Qt. These data do not allow estimation of the magnitude of this discrepancy in these rhinoceros.

The increase in PAP that was observed could be due to increased blood flow or resistance in the arterial segment of the pulmonary bed, increased left ventricular preload, increased left ventricular afterload, or some other cause. In addition to the measurements of PAP and Qt that have been shown to be feasible in white rhinoceros, analysis of the mechanisms behind the increase in PAP will require, at least, measurement of PAOP (which is a surrogate for left atrial pressure) and calculation of pulmonary vascular resistance (PVR). Experiments where $PaCO_2$ and PaO_2 are maintained in a normal range will be necessary to investigate whether hypoxaemia and hypercapnia are necessary intermediates in the cardiovascular effects of etorphine.

Before supplemental etorphine, four of five rhinoceros were hypoxaemic (PaO_2 < 80 mm Hg), and all five were hypercapnic ($PaCO_2$ > 40 mm Hg); this is compatible with considerable pulmonary impairment caused by the initial capture drugs and subsequent recumbency. Since these animals did not run during the darting process, the hypoxaemia and hypercapnia were probably caused by pulmonary depression from the drugs given during the initial chemical restraint. The decrease in PaO_2 and increase in $PaCO_2$ observed after supplemental etorphine are compatible with additional functional impairment of gas exchange and decreased alveolar ventilation (V_A) from the etorphine, although the possibility that these changes were part of ongoing processes initiated by the initial immobilisation cannot be excluded. The increase in PAP with etorphine should increase capillary hydrostatic pressure (Pc) and thus increase the rate of transit of water from the pulmonary capillaries to the interstitium of the lung. If the lymphatic system cannot accommodate this increased load, water will accumulate in the lung. This might impede pulmonary gas diffusion and hence contribute to the decrease in PaO_2 observed after etorphine. Equilibration of haemoglobin (Hb) in erythrocytes with the alveolar gas takes a finite time; the increase in Qt observed with etorphine might decrease the transit time of erythrocytes in the lung sufficiently to prevent such equilibration and thus contribute to the hypoxaemia. Other perturbations of the pulmonary system might also contribute to the hypoxaemia associated with etorphine in white rhinoceros.

The limited number of subjects available made this study underpowered for formally assessing the statistical significance of changes in multiple variables. Also, without suitable control experiments where etorphine was not given, which were not performed for logistical reasons in this study, it is possible that the changes observed after supplemental etorphine might have occurred anyway. In any case, the observations on the effects of supplemental etorphine

reported here are inevitably biased by the etorphine, azaperone, and butorphanol given during initial immobilisation ~1 hour previously. Nevertheless, the temporal relationship of the profound changes that were observed to the time that supplemental etorphine was given allows postulation of a causal relationship with some confidence. The use of supplemental etorphine seemed to be associated with serious adverse cardiopulmonary effects; this argues against the use of etorphine to prolong immobilisation in white rhinoceros and suggests that alternative drugs are preferable (e.g., tranquilisers, sedatives, or dissociative agents).

Conclusions

These preliminary data suggest that etorphine has profound effects on the cardiopulmonary system of white rhinoceros that could compromise them during chemical immobilisation. Further studies are required to confirm the effects of etorphine when it is given alone, and to dissect the mechanisms responsible for these effects on the cardiopulmonary system and their possible relationship to hypoxaemia. Such studies will inform efforts to ameliorate the adverse effects of etorphine and the search for viable alternatives to etorphine.

CHAPTER 3 ETORPHINE-INDUCED HYPOXAEMIA IN WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*): MORE THAN MEETS THE EYE

Abstract

Etorphine immobilisation causes severe physiological derangements in white rhinoceros, including hypoxaemia. I hypothesised that etorphine produces hypoxaemia through mechanisms other than hypoventilation and induces sympathetic upregulation that decreases over time. Six boma-habituated, sub-adult, male white rhinoceros received each of two treatments in random order: etorphine-saline (ES) and etorphine-butorphanol (EB). Rhinoceros were immobilised with 2.6 ± 0.1 µg kg^{-1} (mean ± standard deviation [SD]) of etorphine intramuscularly (IM) by dart and positioned in sternal recumbency (time = 0 minutes [t = 0]). After instrumentation with a custom breathing system with pneumotachometer, they were positioned in left lateral recumbency for insertion of peripheral and Swan-Ganz-type pulmonary arterial catheters (PAC). Baseline data were collected at t = 30, butorphanol (0.026 ± 0.001 mg kg^{-1}) or saline was administered intravenously (IV) at t = 37, and data were collected at t = 40 and 50. A linear mixed effect model was used to identify differences within treatments over time and between treatments at t = 30, 40, and 50. Only data from ES are presented in this chapter. Etorphine caused severe hypoxaemia and hypoventilation (mean arterial oxygen and carbon dioxide partial pressures [PaO_2 and $PaCO_2$] ~30 and 90 mm Hg, respectively), with increased alveolar-arterial oxygen partial pressure difference (P_{A}-aO_2) and shunt fraction (Qs/Qt). Mean pulmonary arterial pressure (mPAP) and pulmonary arterial occlusion pressure (PAOP) were 56-59 mm Hg and 34-37 mm Hg, respectively. Mean thermodilution cardiac output (Qt) was 40-60% higher than predicted allometrically. Mixed venous oxygen partial pressure ($P\bar{v}O_2$) and content ($C\bar{v}O_2$) were low (~20 mm Hg and ~5 mL dL^{-1}, respectively) but increased between t = 30 and 50 (*P* = 0.0068 and 0.0104, respectively). Between t = 30 and 50, V_T decreased 28% (*P* = 0.022). Oxygen consumption (VO_2) was higher than predicted allometrically and decreased 7% between t = 30

and 40 (P = 0.0136). Tremor score decreased between t = 30 and 40 and t = 30 and 50 (P < 0.0001 for both), but muscle tremors occurred throughout immobilisation. Mixed venous lactate concentration decreased 27% between t = 30 and 50 (P = 0.0039). Mean plasma noradrenaline concentrations were substantially higher than basal concentrations in other mammals but did not change significantly over time. Hypoxaemia in etorphine-immobilised white rhinoceros is caused not only by hypoventilation but venous admixture due to a complex array of pathophysiological effects triggered by noradrenaline release. These include increased Qt, which could produce gas diffusion limitation, and tremors, which increase VO_2 and produce a mixed venous hypoxaemia that exacerbates arterial hypoxaemia. Treatment should focus not only on improving ventilation and supplementing oxygen, but on mitigating sympathetic upregulation.

Keywords: Cardiac output, etorphine, hypoxaemia, oxygen consumption, sympathetic, white rhinoceros.

Introduction

The southern white rhinoceros (*Ceratotherium simum ssp. simum*) was classified as 'near-threatened' in 2012 by the International Union for Conservation of Nature and Natural Resources (IUCN) due to 'the continued and increased poaching threat and increasing illegal demand for horn…'.[10] White rhinoceros in southern Africa must be managed for their protection through strategies such as translocation and dehorning, which require capture via chemical immobilisation.[190] Because the death of even a single rhinoceros represents a considerable loss for the species, white rhinoceros are chemically immobilised in the field for treatment of wounds inflicted by other animals and poachers and for various procedures on captive individuals.[191; 192] Thus, chemical immobilisation is critical for conservation of the species.

The temperament of rhinoceros and the rugged terrain in which they live necessitates immobilisation using a potent drug with a rapid onset of action which can be delivered intramuscularly (IM) by dart and pharmacologically reversed. The ultra-potent μ opioid receptor agonist, etorphine, is one of only a few drugs that fulfils these requirements and has been used to immobilise white rhinoceros since the mid-20th century.[15] The etorphine is ordinarily co-administered with a sedative or tranquiliser.[193] The expansion of physiological monitoring (e.g., arterial blood gas and acid-base analysis, invasive systemic arterial pressure [SAP] measurement) of immobilised white rhinoceros exposed the scope of the physiological abnormalities produced by etorphine immobilisation. Arterial hypoxaemia, hypercapnia, respiratory and metabolic (lactic) acidaemia, tachycardia, systemic arterial hypertension, tremors, and increased oxygen consumption (VO_2) have all been documented in white rhinoceros immobilised with etorphine-based protocols.[1-7; 12-14; 57; 59; 60] Maximal exertion and stress triggered by helicopter pursuit and darting in the field could account for these derangements.[1; 5; 7; 59; 60] However, all of these problems have been observed in white rhinoceros darted with etorphine in bomas after a period of habituation to captivity.[2-4; 6; 7] Stress and exertion under these circumstances are minimal (i.e., as low as possible in a captive setting), suggesting that the drugs used for immobilisation are primarily culpable. Boma-habituated white rhinoceros demonstrate hypoxaemia, hypercapnia and respiratory acidaemia, tachycardia, systemic arterial hypertension, and increased VO_2 after etorphine is administered alone, without adjunctive drugs.[2; 3] Thus, it is likely that the aforementioned perturbations are caused mainly by etorphine.

When etorphine is administered to domestic horses, which are phylogenetically related to rhinoceros, they exhibit signs resembling those observed in the etorphine-immobilised white rhinoceros. Hypoxaemia, hypercapnia, acidaemia, sinus tachycardia and other cardiac

dysrhythmias, systemic arterial hypertension, increased cardiac output (Qt), hypertonus of the limbs, full-body tremors, sweating, increased blood glucose concentration, and increased haematocrit are characteristic of horses and ponies immobilised with etorphine.[65-68; 70; 71; 73] Increased sympathetic nervous system (SNS) activity could account for these observations and has been implicated in several publications.[65; 67; 68; 194] Indeed, in horses, plasma noradrenaline concentration increases after etorphine administration, and etorphine-induced tachycardia and hypertension are mitigated by premedication with the β adrenoceptor antagonist, propranolol.[67; 75] Increased muscle activity in etorphine-immobilised white rhinoceros, exhibited as tremors, has been associated with increased adrenaline concentration, lending support to the hypothesis that sympathetic upregulation is responsible for the physiological derangements seen in this species.[7]

Etorphine immobilisation of white rhinoceros is associated with severe hypoventilation (arterial partial pressure of carbon dioxide [$PaCO_2$] as high as 80-100 mm Hg).[2; 17] Nevertheless, the severity of the arterial hypoxaemia observed (arterial partial pressure of oxygen [PaO_2] ~25 mm Hg) and the increased alveolar-arterial oxygen partial pressure difference (P_A-aO_2) suggests that hypercapnia is not solely responsible for the hypoxaemia.[2] Hence, mechanisms other than hypoventilation, such as increased venous admixture, likely contribute to the hypoxaemia.

The pulmonary, cardiovascular, and metabolic effects of etorphine have been studied in white rhinoceros.[2-7] However, a complete picture of its effects requires catheterisation of the pulmonary artery for measurement of pressure and flow in the pulmonary vascular bed and sampling of mixed venous blood, as well as measurement of plasma catecholamine concentrations.

My hypotheses were that 1) mechanisms other than opioid-induced hypoventilation contribute to the hypoxaemia observed in etorphine-immobilised white rhinoceros, and 2)

etorphine immobilisation induces sympathetic upregulation that decreases over the period of immobilisation.

Materials and Methods

This research was approved by the University of Pretoria (UP) Animal Ethics Committee (AEC) (project number V101-15) and the South African National Parks (SANParks) Animal Use and Care Committee (AUCC, reference number 001/16) (Appendix). The management and immobilisation of the rhinoceros were conducted according to the SANParks Standard Operating Procedures for the Capture, Transportation, and Maintenance in Holding Facilities of Wildlife.

Animals

Eight sub-adult (4-5 years old), male white rhinoceros were captured via aerial darting in the Kruger National Park (KNP, 23°49'60 S, 31°30'0 E; altitude: 317 m above sea level), South Africa, and group-housed in Veterinary Wildlife Services (VWS) bomas (i.e., wildlife holding enclosures, dimensions: 10.5 m * 21 m, or 220.5 m^2). Prior to release into the bomas, each rhinoceros underwent physical examination to ensure they were healthy. The rhinoceros were habituated to captivity over at least 1 month and provided water *ad libitum* and a diet of lucerne (*Medicago sativa*) and tef (*Eragrostis tef*) hay. Faeces were removed from the enclosures, water troughs cleaned, and food replaced daily. Trained personnel evaluated the rhinoceros health daily using a scoring system developed by VWS based on the evaluation of: 1) feed intake, 2) volume, consistency, and colour of faeces, and 3) behaviour.[186]

During a pilot study that was conducted prior to the full study described in this thesis, one rhinoceros suffered cardiac arrest during etorphine immobilisation and expired, despite cardiopulmonary resuscitation efforts, including external chest compressions, nasal oxygen supplementation, and administration of intravenous (IV) naltrexone and epinephrine, by the

investigators (including three board-certified anesthesiologists and four wildlife veterinarians). Because gross post-mortem examination did not reveal any pathology that could explain the arrest, the cause of death was deemed likely to be hypoxaemia. A second rhinoceros ingested a foreign object (a small wire), developed signs of esophageal obstruction ('choke') similar to horses. The rhinoceros was immobilised for examination, which revealed rupture and abscessation of the cervical esophagus, necessitating euthanasia. A third rhinoceros was excluded from the study because it scored '6' on the VWS immobilization scoring system (Table 3-3) during two immobilisation attempts and required urgent administration of the opioid antagonist, naltrexone, IV before data collection could be completed. This left a sample size of six rhinoceros for data collection and analysis.

Study Design

A controlled, unblinded, randomised, crossover study design was used. Two treatments (etorphine-saline [ES] and etorphine-butorphanol [EB]) were separated by a washout period of at least 2 weeks. *A priori*, treatment order was assigned randomly by lottery in blocks of two using an online randomiser (www.randomiser.org). Experiments were conducted in the early morning, and atmospheric pressure was measured before each immobilisation (Kestrel Instruments, Boothwyn, Pennsylvania, USA). All drug doses were standard white rhinoceros drug doses used by VWS based on estimated body mass (Table 3-1). Rhinoceros were immobilised in the bomas with etorphine (M99; Voluplex, Mnandi, Centurion, South Africa) delivered IM in the muscles of the nuchal hump using a 3 mL plastic dart with a 60 mm uncollared needle using a compressed air rifle (DAN-INJECT International S.A., Skukuza, South Africa). When a rhinoceros stopped moving and was safe to approach, it was blindfolded while standing, then pulled down into sternal recumbency using ropes (time = 0 minutes [t = 0]). To reduce the

effects of different induction times on physiological data, instrumentation proceeded only if a rhinoceros could be safely positioned in sternal recumbency, which was considered an indicator of equivalent central nervous system (CNS) depression, within 15 minutes of darting. Time between darting and sternal recumbency was recorded.

Table 3-1 Standard doses of drugs used in white rhinoceros by Veterinary Wildlife Services (VWS) based on body mass. All rhinoceros used in this study fell into one of these categories.

Rhinoceros mass (kg)	**Etorphine (mg)**	**Hyaluronidase (IU)**	**Butorphanol (10x etorphine dose, mg)**	**Naltrexone (20x etorphine dose, mg)**
1,000 -1,250	2.5	5,000	25	50
1,250 – 1,500	3.125	5,000	31.25	62.5

IU, International Units

Thirty minutes was allotted for instrumentation once a rhinoceros had been positioned in sternal recumbency. Shortened, cuffed equine endotracheal tubes (KRUSSE Silicone Endotracheal Tube, internal diameter: 28 mm, no. 282270; Jørgen Kruuse A/S, Langeskov, Denmark) were lubricated with water-soluble jelly and inserted into each naris in sternal recumbency; the cuff was inflated to create an air-tight seal. The rhinoceros was then positioned in left lateral recumbency, and instrumentation continued. At t = 30, the first set of data was collected. At t = 37, either butorphanol (Butonil, Wildlife Pharmaceuticals Pty Ltd, White River, South Africa) or an equivalent volume of saline was administered intravenously (IV) in an auricular vein by the same investigator (PB). Data were collected again at t = 40 and t = 50.

After data collection at t = 50, rhinoceros were prepared for recovery by removing all catheters and monitoring devices. Pressure was applied manually to the sites of catheter

placement until adequate haemostasis was judged to have been achieved. Butorphanol was administered IV at this time by PB to the rhinoceros that had not received it previously (i.e., those in treatment ES). Rhinoceros could then be stimulated to stand and walk into a crate for weighing. After weighing, naltrexone (Kyron Laboratories Pty Ltd, Johannesburg, South Africa) was administered IV, and the rhinoceros were released back into the bomas.

Instrumentation and Data Collection

Both endotracheal tubes were connected to an Exercise Physiology System with PowerLab 8/30 data acquisition hardware (ML870, ADInstruments Pty Ltd, New South Wales, Australia) and LabChart 7 data acquisition software with Metabolic Module (version 7.3.8, ADInstruments) installed on a laptop computer (Figure 6-5). The tubes were connected to the system at the margins of the nares via Y pieces. Each Y piece housed a one-way inspiratory valve, which allowed inspiration of ambient air, and a one-way expiratory valve (Two-Way Non-Rebreathing Valve, Series 2730; Hans Rudolph, Inc., Shawnee, Kansas, USA). Expired air from both Y pieces entered a T piece via plastic breathing tubes, then, via a common tube, entered a 4.7 L gas mixing chamber (MLA245; ADInstruments Pty Ltd). Expired gas temperature (°C) was measured in the chamber by a thermistor (Thermistor Temperature Sensor, MLT415/M, ADInstruments Pty Ltd) connected to a temperature pod (ML309, ADInstruments Pty Ltd). Gas from the chamber was then directed through a pneumotachometer (Respiratory Flow Head 1000L, MLT300L, ADInstruments Pty Ltd) and into the atmosphere, with the exception of 1 minute at the beginning of each sampling point, when a Douglas bag was attached to the pneumotachometer for collection of mixed expired gas. The pneumotachometer was connected to a differential pressure transducer (Spirometer, ML141, ADInstruments Pty Ltd), which was connected to the PowerLab 8/30 (ADInstruments Pty Ltd). The pneumotachometer was capable

of achieving laminar flow at flow rates up to 1000 L minute^{-1} and was calibrated, using a 3 L calibration syringe, each morning according to the manufacturer's instructions before rhinoceros immobilisation. Mixed expired carbon dioxide partial pressure ($P_{\bar{E}}CO_2$, mm Hg) and mixed expired oxygen fraction ($F_{\bar{E}}O_2$, %) were measured from the Douglas bag with the Cardiocap/5 (Datex-Ohmeda, GE Healthcare, Helsinki 00510, Finland), which auto-calibrated upon start-up with ambient air and was also calibrated with a carbon dioxide (CO_2) canister. This monitor was also used to analyse end-tidal oxygen partial pressure (P_EO_2) and end-tidal carbon dioxide partial pressure (P_ECO_2) via a sampling port in one of the equine endotracheal tubes just outside the naris. For further details, see Chapter 6.

After aseptic skin preparation, a 22 standard wire gauge (SWG), 2.5 cm over-the-needle IV catheter (Nipro Medical Corporation, Bridgewater Township, New Jersey, USA) was inserted into a medial auricular artery and connected by noncompliant tubing to a transducer (Deltran II pressure transducers, DPT-200, Utah Medical Products Inc., Midvale, Utah, USA) zeroed to atmospheric pressure at the level of the *manubrium sterni*. The transducer was connected to a blood pressure amplifier (BP Amp, FE117, ADInstruments Pty Ltd), which was connected to the PowerLab 8/30 (ADIntruments).

After aseptic skin preparation and draping over the angle of the mandible, a percutaneous sheath introducer (11 French [Fr], Super Arrow-Flex Sheath Set, Teleflex, Morrisville, North Carolina, USA) was inserted into the linguofacial branch of the right jugular vein as described previously.[17] Briefly, under ultrasonographic (Hitachi Noblus ultrasound system fitted with a C25 convex 5 – 1 MHz transducer, Hitachi Aloka Medical, Wallingford, Connecticut, USA) guidance a needle (Vasofix Braunüle, B Braun South Africa, Johannesburg, Gauteng, South Africa) was inserted through a small stab incision and into the vein. Once venous blood was

observed in the needle hub, a wire guide was inserted through it. With the wire guide *in situ*, the needle was removed, and a dilator and introducer were inserted over the wire guide. After the wire and dilator were removed, a sterile, custom-built, 200 cm, 7 Fr (2.33 mm), Swan-Ganz-type thermodilution pulmonary arterial catheter (PAC, Gaeltec Devices, Dunvegan, Isle of Skye, Scotland) was then inserted through the introducer and down the jugular vein (Figure 6-1, Box 6-1). The distal port of the PAC was connected by noncompliant tubing to another Deltran II pressure transducer (Utah Medical Products) that was zeroed to atmospheric pressure at the level of the *manubrium sterni* and connected to the amplifier (ADInstruments Pty Ltd) and PowerLab 8/30 (ADInstruments Pty Ltd). The PAC was passed through the introducer, and the balloon was inflated with a specific volume of air. This volume was determined just prior to each immobilisation by injecting air with a syringe into the pilot balloon until the moment the balloon inflated; the volume of air injected was recorded. The tip of the PAC was passed through the right heart and into the pulmonary artery while observing the characteristic pressure waveforms on a laptop computer. Once a pulmonary arterial pressure (PAP) waveform was observed, the PAC was advanced further until the balloon wedged in a branch of the pulmonary artery and a pulmonary arterial occlusion pressure (PAOP) waveform was observed on the monitor. A PAOP was recorded within 10 seconds of wedging, and the balloon was deflated. The PAC was left with the tip in the pulmonary artery for subsequent pressure measurements. A thermistor at the tip measured pulmonary artery temperature (°C). Values for mean PAP (mPAP), PAOP, and mean systemic arterial pressure (mSAP) were confirmed by analysing the pulmonary and SAP waveforms displayed by the LabChart 7. For further details, refer to Chapter 6.

Cardiac output was measured by thermodilution (PM-9000 Vet Veterinary Portable Multi-Parameter Patient Monitor, ShenZhen Mindray Bio-Medical Electronics Co., Nanshan,

ShenZhen, China). The thermistor in the PAC had the same properties as the thermistor used in ARROW Balloon Thermodilution catheters (Teleflex, Morrisville, North Carolina, USA). The appropriate computation constant for that thermistor (0.308) and an injectate volume of '6 mL' were entered into the monitor. Sixty mL (10 times the volume entered into the monitor) of ice-cold 0.9% saline was hand-injected over < 3 seconds through the side port of the introducer into the linguofacial vein at end-expiration. Three to five sequential measurements were recorded at each sampling point, and each was multiplied by a factor of 10 to obtain Qt in L minute^{-1}. The median value was used for analysis.

At each sampling point, arterial and mixed venous blood samples were collected simultaneously from the systemic arterial catheter and the distal port of the PAC, respectively. First, at least 10 times the volume of each catheter lumen was withdrawn and discarded. Blood samples (1 mL each) were then collected anaerobically into 1 mL heparinised syringes, put on ice, and analysed immediately after sampling with a point-of-care analyser (epoc Blood Analysis System, Siemens Medical Solutions, Inc., Malvern, Pennsylvania, USA) which produced results in less than 1 minute. Values for pH and blood gas tensions were corrected to pulmonary artery temperature. A portion of each sample was spun down to obtain packed cell volume (PCV), which was used to calculate haemoglobin concentration ([Hb]) (Table 3-4, Figure 6-6). Blood was also collected from an auricular vein into ethylene-diamine-tetra-acetic acid (EDTA) tubes, which were immediately put on ice and spun down in a cold centrifuge at 2,500 revolutions minute^{-1} for 10 minutes. Plasma was pipetted into cryotubes (Greiner Bio-One, Frickenhausen, Baden-Württemberg 72636, Germany), snap-frozen in liquid nitrogen, and stored at -80°C. This plasma was used to measure catecholamine concentrations with high-performance liquid chromatography-electrochemical detection (HPLC-ECD, Agilent 1200 HPLC, Agilent

Technologies, Santa Clara, California, USA) using previously published methods.[195] For further details, see Chapter 6.

Rectal temperature was measured using a thermometer inserted deep into the rectum against the rectal wall. Heart rate was measured via thoracic auscultation and confirmed with the SAP waveform. Skeletal muscle tremors and immobilisation depth were scored by a single experienced, unblinded observer (PB) using scoring systems (Table 3-2, Table 3-3).

Table 3-2 Tremor scoring system for etorphine-immobilised white rhinoceros developed by Veterinary Wildlife Services (VWS).

Score	Description
1	No visible tremors
2	Mild tremors – resulting in minor leg and foot movement
3	Slight tremors – resulting in minor shoulder, chest and severe leg and foot movement
4	Moderate tremors – resulting in severe shoulder, chest, leg and foot movement
5	Severe tremors – resulting in whole body and head movement

Table 3-3 Immobilisation scoring system for etorphine-immobilised white rhinoceros developed by Veterinary Wildlife Services (VWS).

Score	Description
1	No effect
2	Cannot handle safely (standing)
3	Can handle safely (standing)

Table 3-3, continued

4	Recumbent, ear movements, tail curled
5	Recumbent, fully relaxed, no ear movements, tail relaxed
6	Recumbent, excessive depth, respiratory rate less than 3 breaths minute^{-1}

All data were collected by PowerLab 8/30 data acquisition hardware (ML870; ADInstruments Pty Ltd) and transmitted to a laptop computer with LabChart 7 data acquisition software (ADInstruments Pty Ltd). Values for mPAP, PAOP, and mean systemic arterial pressure (mSAP) were measured and confirmed by analysing the pulmonary and systemic arterial pressure waveforms displayed by LabChart 7 (Chapter 6). Expired minute ventilation at body temperature and pressure, saturated with water vapour (V_{EBTPS}) and respiratory rate (f_R) were measured using the expiratory flow waveform displayed by LabChart 7 (Box 6-2). V_{EBTPS} was converted to expired minute ventilation, standard temperature and pressure, dry (V_{ESTPD}) (Table 3-4). Mixed expired carbon dioxide partial pressure was divided by P_B to calculate mixed venous carbon dioxide fraction ($F\bar{E}CO_2$), which was used, along with $F\bar{E}O_2$, to calculate oxygen consumption (VO_2, L minute^{-1}) (Table 3-4, Box 6-7). V_{ESTPD} was also used to calculate carbon dioxide production (VCO_2, L minute^{-1}, Table 3-4). Pulmonary vascular resistance (PVR), stroke volume (SV), alveolar-arterial oxygen partial pressure difference (P_{A}-aO_2), arterial and mixed venous oxygen content (CaO_2 and $C\bar{v}O_2$), tidal volume (V_T), physiological dead space ventilation (V_{DPHYS}), VO_2, VCO_2, oxygen delivery (DO_2), oxygen extraction ratio (OER), pulmonary end-capillary oxygen content ($Cc'O_2$), and shunt fraction (Qs/Qt) were calculated using equations in Table 3-4.

Table 3-4 Equations for calculated variables in six boma-habituated, sub-adult, male white rhinoceros.

Calculated Variable	Equation
PVR (mmHg * minute L^{-1})	(mPAP – PAOP)/Qt
SV (mL $beat^{-1}$ kg^{-1})	Qt/f_H
P_AO_2 (mm Hg)	$F_IO_2 * (P_B - P_{WV}) – (PaCO_2/1)$
P_A-aO_2 (mm Hg)	$P_AO_2 – PaO_2$
[Hb] (g dL^{-1})	(0.2528 * PCV) + 4.013[a]
CaO_2 (mL dL^{-1})	([Hb] * 1.39 * SaO_2[a]) + (PaO_2 * 0.003)
$C\bar{v}O_2$ (mL dL^{-1})	([Hb] * 1.39 * $S\bar{v}O_2$[a]) + ($P\bar{v}O_2$ * 0.003)
$Cc'O_2$ (mL dL^{-1})	([Hb] * 1.39 * SO_2[b]) + (P_AO_2 * 0.003)
V_{ESTPD} (mL $minute^{-1}$ kg^{-1})	(273/310)[(P_B – 47)/760] * V_{EBTPS}
V_T (mL $breath^{-1}$ kg^{-1})	V_{ESTPD}/f_R
V_{DPHYS} (mL $minute^{-1}$ kg^{-1})	[($PaCO_2$ – $P_{\bar{E}}CO_2$)/$PaCO_2$]* V_T – 0.3[d]
DO_2 (mL $minute^{-1}$ kg^{-1})	CaO_2 * Qt
VO_2 (mL $minute^{-1}$ kg^{-1})	(V_I[c] * F_IO_2) - (V_E * F_EO_2)
VCO_2 (mL $minute^{-1}$ kg^{-1})	V_{ESTPD} * (F_ECO_2 – F_ICO_2)
OER (%)	VO_2/DO_2
Qs/Qt (%)	($Cc'O_2$ – CaO_2)/($Cc'O_2$ – $C\bar{v}O_2$)

PVR, pulmonary vascular resistance; mPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; Qt, cardiac output; SV, stroke volume; f_H, heart rate; P_AO_2, alveolar oxygen partial pressure; F_IO_2, inspired oxygen fraction; P_B, barometric pressure; P_{WV}, saturated water vapour pressure; $PaCO_2$, arterial carbon dioxide partial pressure; P_A-aO_2, alveolar-arterial oxygen partial pressure difference; PaO_2, arterial oxygen partial pressure;

Table 3-4, continued

[Hb], haemoglobin concentration; PCV, packed cell volume; CaO_2, arterial oxygen content; SaO_2, arterial oxyhaemoglobin saturation; $C\bar{v}O_2$, mixed venous oxygen content; $S\bar{v}O_2$, mixed venous oxyhaemoglobin saturation; $P\bar{v}O_2$, mixed venous oxygen partial pressure; $Cc'O_2$, pulmonary end-capillary oxygen content; SO_2, oxyhaemoglobin saturation (general); V_{EBTPS}, minute ventilation, body temperature and pressure standard; V_T, tidal volume; V_{ESTPD}, minute ventilation, standard temperature and pressure, dry; f_R, respiratory rate; V_{DPHYS}, physiological dead space ventilation; $P_{\bar{E}}CO_2$, mixed expired carbon dioxide partial pressure; DO_2, oxygen delivery; VO_2, oxygen consumption; V_I, inspired volume; V_E, minute ventilation (or expired volume); F_EO_2, expired oxygen fraction; VCO_2, carbon dioxide production; F_ECO_2, expired carbon dioxide fraction; F_ICO_2, inspired carbon dioxide fraction; OER, oxygen extraction ratio; Qs/Qt, shunt fraction.

[a] Refer to Chapter 6 for further details.

[b] Values for $PaCO_2$ and arterial pH were used to calculate SO_2 in the formula for $Cc'O_2$.

[c] V_I was not necessarily equivalent to V_E because VO_2 did not necessarily equal VCO_2 (i.e., the respiratory exchange ratio [RER] was not necessarily 1). Therefore, the Haldane transformation was used to determine V_I.[196] Refer to Chapter 6 for further details.

[d] 0.3 L was subtracted to account for the volume of the tubes external to the nares.

Statistical Analysis

After performing a Shapiro-Wilk test to confirm normality of the data, a paired t-test was used to compare body mass of the rhinoceros, etorphine dose, time to sternal recumbency, and atmospheric temperature in the two treatments. Residuals were calculated, and the Shapiro-Wilk test was used to confirm that the residuals were normally distributed.

Statistical analysis was performed using commercial software (JMP 14.0.0, SAS Institute, Inc., Cary, North Carolina, USA). A linear mixed effect model was constructed, with each of the physiological variables designated as the response (‘dependent’) variables. Time, treatment (or ‘phase’), and the interaction of time and treatment (‘phase’) were designated as fixed effects, and rhinoceros identification number (ID) and rhinoceros ID nested within treatment were designated

as random effects. *A priori*, mPAP, PAOP, Qt, PaO_2, mixed venous oxygen partial pressure ($P\bar{v}O_2$), DO_2, VO_2, and noradrenaline concentration were designated as the primary outcomes. The remaining variables were designated as secondary outcomes. Alpha was set at 0.05 for the eight primary outcomes, and 0.05/20 = 0.0025 for the 18 secondary outcomes (a Bonferroni correction for multiple comparisons). For each variable, the 36 residuals (three sampling points * two treatments * six rhinoceros) were calculated, and the Shapiro-Wilk test was used to confirm that the residuals were normally distributed. Residuals for PVR, SV, $P_A\text{-}aO_2$, f_R, noradrenaline concentration, and OER were not normally distributed; thus, the data for these variables was log-transformed, and residuals were re-tested to confirm normality. Parameter estimates for all variables are listed in Table 6-2.

Results

The rhinoceros' body mass during the saline treatment was 1145 ± 73 kg (mean ± standard deviation [SD]). The etorphine dose administered was 2.6 ± 0.1 µg kg^{-1}. Time to sternal recumbency was 12.4 ± 2.0 minutes.

Mean ± SD for all variables are listed in Table 3-5. At t = 50, $P\bar{v}O_2$, CaO_2, and $C\bar{v}O_2$ were 19%, 20%, and 37% higher than at t = 30 (*P* = 0.0018, 0.0109, and 0.0104, respectively). However, after Bonferroni correction, the increases in CaO_2 and $C\bar{v}O_2$ lost significance. At t = 50, V_T was 28% lower than at t = 30 (*P* = 0.022); this lost significance after Bonferroni correction. At t = 40, VO_2 was 7% lower than at t = 30 (*P* = 0.0136); however, by t = 50, it was 17% higher than at t = 30 (*P* = 0.0082). Tremor score was lower at t = 40 and 50 than at t = 30 (*P* < 0.0001 for both). At t = 50, mixed venous lactate concentration was 27% lower than at t = 30 (*P* = 0.0039); this lost significance after Bonferroni correction.

Concentrations of adrenaline and dopamine were below than the lower limit of detection of the assay in most samples and were thus not analysed. Noradrenaline concentrations were measurable at all sampling points during each immobilisation but did not change significantly over time.

None of the other variables changed significantly over time.

Table 3-5 Physiological variables in six boma-habituated, sub-adult, male white rhinoceros immobilised with etorphine intramuscularly (IM). After positioning in sternal recumbency (time = 0 minutes [t = 0]), data were collected at t = 30 (baseline), 40, and 50. Saline placebo or butorphanol was administered intravenously (IV) at t = 37. Blood gas partial pressures were corrected to pulmonary artery temperature. A linear mixed effect model was constructed to identify differences within treatments over time and between treatments at t = 30, 40, and 50. This table shows differences within EB over time. The primary outcomes are bold. †Significantly different from value at t = 30.

	t = 30		**t = 40**		**t = 50**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
mPAP (mmHg)	59	8	56	10	56	8
PAOP (mmHg)	37	9	34	8	34	6
mSAP (mmHg)	150	25	145	20	145	17
Qt (mL minute^{-1} kg^{-1})	81	26	80	22	73	13
PVR (mmHg * minute L^{-1})	0.26	0.07	0.26	0.10	0.27	0.05
f_H (beats minute^{-1})	121	21	121	16	116	21
SV (mL beat^{-1} kg^{-1})	0.7	0.1	0.7	0.2	0.6	0.0

Table 3-5, continued

T (PA, °C)	38.4	0.6	38.3	0.6	38.2	0.6
T (rectal, °C)	38.5	0.4	38.6	0.6	38.6	0.6
PaO_2 (mmHg)	29	3	30	3	31	3
$PaCO_2$ (mmHg)	92	8	93	11	88	8
$P\bar{v}O_2$ (mmHg)	20	3	22	4	24†	3
P_{A}-aO_2 (mmHg)	24	8	22	10	26	7
CaO_2 (mL dL^{-1})	9.7	3.2	10.4	3.2	11.6	2.8
$C\bar{v}O_2$ (mL dL^{-1})	4.8	2.5	5.6	2.2	6.5	2.3
V_{EBTPS} (mL $minute^{-1}$ kg^{-1})	100.1	19.9	95.4	15.1	90.9	10.1
f_R (breaths $minute^{-1}$)	5.6	2.4	6.3	2.6	7.4	2.2
V_T (mL $breath^{-1}$ kg^{-1})	19.6	7.0	16.7	5.1	14.1	3.6
V_{DPHYS} (mL $minute^{-1}$ kg^{-1})	49.7	12.2	50.5	11.8	50.2	9.8
VO_2 (mL $minute^{-1}$ kg^{-1})	4.8	0.9	4.5†	0.6	5.4†	0.6
VCO_2 (mL $minute^{-1}$ kg^{-1})	4.2	1.1	3.9	0.7	4.6	0.5
DO_2 (mL $minute^{-1}$ kg^{-1})	7.3	1.7	8.1	3.0	8.2	1.6
OER (%)	69.5	22.1	63.2	31.7	68.8	22.0
Qs/Qt (%)	60.1	9.7	54.5	5.7	57.9	6.9
Noradrenaline (ng mL^{-1})	233.5	74.6	220.7	60.0	212.4	68.5
Tremor score	2.8	0.4	2.0†	0.0	2.0†	0.0
Lactate (mmol L^{-1})[a]	2.1	1.8	1.8	1.5	1.6	1.3
Hb (g dL^{-1})	15.6	0.4	15.1	0.5	15.1	0.4

Table 3-5, continued

SD, standard deviation; mPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; mSAP, mean systemic arterial pressure; Qt, cardiac output; PVR, pulmonary vascular resistance; f_H, heart rate; SV, stroke volume; T, temperature; PA, pulmonary artery; PaO_2, arterial oxygen partial pressure; $PaCO_2$, arterial carbon dioxide partial pressure; $P\bar{v}O_2$, mixed venous oxygen partial pressure; $P_A\text{-}aO_2$, alveolar-arterial oxygen partial pressure difference; CaO_2, arterial oxygen content; $C\bar{v}O_2$, mixed venous oxygen content; V_{EBTPS}, minute ventilation, body temperature and pressure, saturated with water vapour; f_R, respiratory rate; V_T, tidal volume; V_{DPHYS}, physiological dead space ventilation; VO_2, oxygen consumption; VCO_2, carbon dioxide production; DO_2, oxygen delivery; OER, oxygen extraction ratio; Qs/Qt, shunt fraction; Hb, haemoglobin.

[a] Mixed venous lactate.

Discussion

Consistent with previous work, etorphine immobilisation uniformly produced severe hypoxaemia (PaO_2 ~30 mm Hg) that persisted throughout the immobilisation.[2] Also consistent with previous work, hypoventilation played a significant role in the hypoxaemia; $PaCO_2$ was > 80 mm Hg throughout the immobilisation and reached > 100 mm Hg in some rhinoceros.[2] Furthermore, mean V_{EBTPS} was ~100, 95, and 91 mL $minute^{-1}$ kg^{-1} at t = 30, 40, and 50, which is 27-40% lower than expected for conscious, resting white rhinoceros of similar size; the allometric equation $0.5 * M_b^{(0.8)}$ (where M_b = body mass in kg) predicts that mean V_{EBTPS} should be ~127 ± 1.6 mL $minute^{-1}$ kg^{-1}.[197] Hypoventilation causes hypoxaemia by causing an increase in alveolar CO_2, which dilutes alveolar oxygen.[198]

However, the rhinoceros also had a mildly to moderately increased $P_A\text{-}aO_2$, with some $P_A\text{-}aO_2$ values > 30 mm Hg, as well as very high Qs/Qt (mean of 55-60%), demonstrating that hypoventilation was not the only mechanism responsible for the hypoxaemia and that venous admixture must have also contributed. Venous admixture is defined as the degree of admixture of mixed venous blood with pulmonary end-capillary blood that would be required to produce

the observed difference between PaO_2 and pulmonary end-capillary oxygen partial pressure ($Pc'O_2$). Diffusion limitation, ventilation:perfusion (V:Q) mismatching (V:Q < 1), and right-to-left shunt (caused by either shunting of blood past completely collapsed alveoli, i.e., V:Q = 0, or anatomic shunt, the latter being less likely in these rhinoceros given that congenital cardiac defects are rare in other species) can all contribute to venous admixture.

Diffusion limitation could be explained by either decreased erythrocyte transit time through pulmonary capillaries or development of interstitial pulmonary oedema or both. Increased pulmonary blood flow (right-sided Qt) reduces erythrocyte transit time through the pulmonary capillaries, shortening the duration of erythrocyte exposure to alveolar gas and decreasing the time for equilibration of oxygen.[198] Interstitial pulmonary oedema increases the diffusion path for oxygen across the alveolar-capillary barrier and occurs when fluid flux (J_v) from the capillary lumen to the interstitial space increases without an increase in lymphatic drainage sufficient to accommodate this increase. Clinically, the most common reasons for an increase in J_v are increased capillary hydrostatic pressure (Pc), damage to the alveolar-capillary membrane (which increases conductivity, K_f, and decreases the Staverman reflection coefficient, σ), or decreased capillary oncotic pressure (Πg) (Chapter 1).[177] Increased Pc or damage to the alveolar-capillary membrane were the most likely mechanisms in these rhinoceros.

As described in Chapter 1, arterial hypoxaemia occurs in horses during high-intensity exercise.[168] Substantial haemodynamic changes in horses during high-intensity exercise contribute to this hypoxaemia by producing diffusion limitation.[116] These haemodynamic changes resemble those observed in etorphine-immobilised horses and rhinoceros.[168; 171] Thus, studies in the exercising horse provide insight into how etorphine might produce hypoxaemia through diffusion limitation. One of the most substantial haemodynamic changes in the

exercising horse is the dramatic increase in Qt, which occurs primarily due to an increase in f_H rather than SV.[113; 168; 178] Pulmonary hypertension has also been documented in horses during maximal exertion; the increase in Qt could account for this observation.[168; 171] An increase in Pc could result and has been documented in horses during maximal exertion using the pulmonary arterial occlusion technique.[168] Large increases in J_v across the capillary-alveolar barrier have also been documented in horses during exercise, based on Qt measured using the Fick principle and calculations of blood volume changes across the lungs; furthermore, ultrastructural examination of horses' lungs has demonstrated interstitial oedema.[178; 183] If Pc and J_v continues to increase, the interstitium will eventually be unable to accommodate the excess fluid, resulting in alveolar oedema and production of frothy sputum.[177] Very high (75-100 mm Hg) pulmonary transmural capillary pressures (Ptm) can cause stress failure of pulmonary capillaries, resulting in haemorrhage into the alveoli and production of sanguinous sputum or even frank blood.[181; 183; 184] Exercise-induced pulmonary haemorrhage (EIPH) in racehorses, characterised by epistaxis during or after a race, appears to result from such stress failure. The Ptm required to cause stress failure of pulmonary capillaries is, however, species-specific, and this value is unknown in the rhinoceros.[184; 185] In rabbits, Ptm of only ~40 mm Hg is required to disrupt the alveolar-capillary membrane.[199] An increase in PAOP, an estimate of left atrial pressure, has also been documented in exercising horses as well as humans.[168] This increase could reflect the increase in Qt as well as an increase in left ventricular end-diastolic pressure (LVEDP) due to incomplete relaxation of the left ventricle.[200] An increased PAOP could increase pulmonary venous pressure and contribute to an increase in Pc.[200]

Haemodynamic measurements in the rhinoceros were consistent with those seen in exercising horses and support diffusion limitation as a cause of the hypoxaemia. Mean Qt was

~70-80 mL minute^{-1} kg^{-1} throughout the study period, 40-60% more than predicted by the allometric equation, 187 * Mb$^{(0.81)}$ (~50 mL minute^{-1} kg^{-1}) for resting rhinoceros of similar mass.[122] The increase in Qt was likely due at least in part to the tachycardia observed throughout the immobilisation (~90-150 beats minute^{-1}, substantially higher than f_H of ~39 beats minute^{-1} recorded in conscious, resting white rhinoceros).[44] Mean pulmonary arterial pressure was ~53-58 mm Hg in the rhinoceros, with a maximum mPAP of 68 mm Hg recorded in a rhinoceros at t = 30. Normal resting mPAP is not known in rhinoceros, but in resting horses, mPAP is ~32 mm Hg.[168; 170] Pulmonary arterial occlusion pressure was ~34-37 mm Hg in the rhinoceros, with a maximum 48 mm Hg recorded in a rhinoceros at t = 30; PAOP is ~20 mm Hg (almost 50% lower) in resting horses and increases during galloping to values similar to those measured in the etorphine-immobilised white rhinoceros.[170] Protective mechanisms (i.e., the retention of water in the capillary by Πc, and lymphatic drainage) can prevent interstitial oedema up to PAOP of ~20-25 mm Hg in humans.[177] With PAOP almost twice as high in these rhinoceros, it is likely that interstitial oedema or even, occasionally, capillary stress failure, occurs in the etorphine-immobilised rhinoceros; oedema or stress failure would explain the reports from wildlife veterinarians of foam, sometimes tinged with blood, at the nares of rhinoceros that died during immobilisation (Prof. Leith Meyer, personal communication). Mean systemic arterial pressure was on average 150 mm Hg, about 50% higher than mSAP recorded in resting horses; this mSAP is consistent with 1) the increase in Qt and 2) the 'arterial baroreceptor reflex' and the resetting of baroreceptors to maintain a higher mSAP during exercise, as described in Chapter 1.[105; 156] Normal mPAP, PAOP, and mSAP for conscious, resting rhinoceros are unknown, but because blood pressure generally does not scale systematically with body mass, it seems reasonable to postulate that mPAP, PAOP, and mSAP in these rhinoceros were abnormally

high.[201] These values were recorded over half an hour after etorphine injection hence it is likely that during the instrumentation period, pressures were even higher. Pulmonary vascular resistance in these rhinoceros was within the normal reference range for horses.[172] This observation is consistent with the observation that in exercising horses, PVR reaches its lowest point during moderate exercise, demonstrating that the increase in mPAP is caused by the increase in Qt and that vasoconstriction does not appear to be the primary mechanism responsible for the increase Qt, at variance to results in etorphine-immobilised goats.[76; 172]

Ventilation:perfusion mismatching likely also plays a role in the hypoxaemia observed in etorphine-immobilised white rhinoceros. A decrease in PaO_2 and increase in P_A-aO_2 and Qs/Qt develop in the laterally recumbent, anaesthetised horse.[202-206] Even when horses are conscious or sedated, lateral recumbency causes some decrease in PaO_2, demonstrating the effect of posture on pulmonary function in this species.[207; 208] Body mass correlates strongly and positively with PaO_2, and shorter, stockier horses with round abdomens (a body conformation similar to that of the white rhinoceros) develop higher P_A-aO_2 than taller horses with flat abdomens.[209; 210] A decrease in functional residual capacity (FRC) due to cranial displacement of the diaphragm and resultant atelectasis of the dependent lung creates areas of consolidation on radiographs and computed tomographic [CT]) images in anaesthetised, laterally recumbent horses and ponies.[205; 211; 212] The high Qs/Qt calculated in these rhinoceros have been documented during one-lung ventilation in other species.[213] Given the body mass and confirmation of the white rhinoceros, it is plausible that a significant portion of the dependent lung is not ventilated in lateral recumbency during etorphine-immobilisation; this is consistent with the distribution of ventilation in the white rhinoceros documented using electrical impedance tomography (EIT, Dr. Martina Mosing, personal communication).

A decrease in $P\bar{v}O_2$ (and subsequently, $S\bar{v}O_2$ and $C\bar{v}O_2$) can exacerbate arterial hypoxaemia. These rhinoceros had $P\bar{v}O_2$ well below the normal value in resting horses.[105] Low $P\bar{v}O_2$ can result from 1) decreased DO_2 or 2) increased VO_2. Either a decrease in DO_2 or an increase in VO_2 increases the oxygen extraction ratio (OER, or VO_2/DO_2), which in these rhinoceros far exceeded the normal range of 20-30%.[214; 215] Decreased DO_2, the product of Qt and CaO_2, can result from either decreased Qt (not observed in the rhinoceros) or decreased CaO_2 (which was observed). Oxygen consumption in these rhinoceros predicted by the allometric equation $11.6 * M_b^{(0.76)}$ is ~2.1-2.2 mL $minute^{-1}$ kg^{-1}.[216] The values for VO_2 calculated in this study were more than twice this rate. (Although VO_2 was lower at t = 40, it was still higher than predicted allometrically.) Increased VO_2 can be caused by exercise, tremors/seizures, shivering, and hyperthermia (discussed below).[2; 105; 217; 218] The rhinoceros in this study developed severe limb tremors as well as milder tremors of the shoulder and thorax. de Lange et al. (2017) found that tremor intensity correlated negatively with PaO_2 in both boma-habituated and free-ranging white rhinoceros immobilised with etorphine and azaperone.[7] The decrease in tremor score between t = 30 and 40 could have caused the decrease in VO_2 at t = 40. Tremor score also decreased between t = 30 and 50, yet despite this decrease, VO_2 at t = 50 was actually higher than at t = 30. It is possible that the level of etorphine-induced CNS depression was (subclinically) decreasing by t = 50 (slightly > 1 hour after darting) and rhinoceros were arousing; this could have caused sympathetic stimulation that increased global VO_2. This may have masked a decrease in VO_2 at t = 50 due to tremor abatement. Despite the increase in VO_2 at t = 50, which should have decreased $P\bar{v}O_2$, $P\bar{v}O_2$ increased at t = 50. This may have been due to the (non-significant) increase in DO_2. Tachycardia increases myocardial VO_2, suggesting that the heart, one of the most metabolically active tissues in the body, played a role (albeit likely small

in comparison to the skeletal muscles) in increasing VO_2 in the rhinoceros.[219] Citino and Bush (2007) recorded a mean rectal temperature of ~36.8°C in healthy, resting, standing, unstressed, and unmedicated white rhinoceros.[44] The rhinoceros in this study had mean rectal and pulmonary artery temperatures > 38°C throughout the study period. Hyperthermia can increase VO_2 (although rectal and pulmonary artery temperatures did not decrease at t = 40 and then increase at t = 50, as VO_2 did).[218] The rhinoceros may have been hyperthermic due to 1) tremors, because contracting muscle generates heat (the reason why hypothermic animals shiver), or 2) an etorphine-induced global increase in their metabolic rate.[218; 220; 221] Hyperthermia can in turn cause other physiological derangements (e.g., tachycardia).[222]

Basal catecholamine concentrations have been measured in a variety of mammalian species and vary with age, species, blood sampling site, and other factors.[223] However, in most mammals, basal concentrations measured using the same assay used in this study are far lower (< 1 ng mL^{-1}) than those measured in these rhinoceros.[223] One study in healthy, unstressed, supine, young adult humans reported a mean noradrenaline concentration of 1.06 nmol L^{-1}, or 0.176 ng mL^{-1}.[224] Mean noradrenaline concentration in these rhinoceros was more than 200 ng mL^{-1}. Even humans with phaeochromocytoma diagnosed on post-operative histopathology do not achieve plasma nordarenaline concentrations as high as the concentrations observed in these rhinoceros.[225] Thus, although normal basal noradrenaline concentration in the white rhinoceros is unknown, it seems reasonable to conclude that etorphine immobilisation causes a profound increase in plasma noradrenaline, on the order of several hundred-fold more than in other mammals. The precise mechanism responsible for the increase in noradrenaline is unclear. Hypoxaemia and hypercapnia can increase plasma concentrations in other mammals, but this increase is relatively minor when compared to the concentrations measured in these rhinoceros,

suggesting another mechanism is responsible for the nordarenaline release.[226; 227] It is possible that the white rhinoceros simply responds with greater sympathetic outflow to hypoxaemia and hypercapnia than other mammals. However, as discussed in Chapter 1, a large body of research, primarily from rats, demonstrates that opioids are capable of inducing catecholamine release and causing clinical signs consistent with sympathetic upregulation, such as tachycardia and hypertension, when injected into discrete brain sites.[8] In conscious rats, intracerebroventricular or intracisternal injection, or injection into the paraventricular nucleus (PVN) or medial preoptic nucleus (POM) of the hypothalamus of low doses of μ opioid receptor agonists consistently increases sympathetic outflow.[80; 95-99] Indeed, opioid receptors have been identified in the hypothalamus.[89] The hypothalamus and the rostral ventromedial medulla (RVLM), the final site of output to the intermediolateral (IML) cell column of the thoracolumbar spinal cord, where the cell bodies of preganglionic sympathetic neurons are located, communicate; the hypothalamus also makes direct connections to the IML cell column.[81; 87] Part of the hypothalamus lies outside the blood-brain barrier and could theoretically be rapidly exposed to a parenterally administered, exogenous opioid like etorphine, then stimulate the RVLM, increasing sympathetic outflow.[77] Cardiovascular and respiratory autonomic effects vary depending on a number of factors such as species, dose of opioid injected, the brain region injected, route of injection (i.e., intraventricular vs. injection into specific brain nuclei, systemic vs. central administration), receptor selectivity and affinity, ventilation (spontaneously vs. mechanically ventilated), and state of consciousness (i.e., anaesthetised vs. conscious).[9] Thus, although extrapolation between species should be done with caution, a direct sympathomimetic effect of etorphine on the white rhinoceros seems plausable. The haemodynamic changes and hypermetabolism are consistent with the high plasma noradrenaline concentrations measured in the rhinoceros. The tremors observed in etorphine-

immobilised white rhinoceros may result from catecholamine agonism of ß$_2$ receptors in skeletal muscle. de Lange et al. (2017) found that tremor intensity measured using activity loggers in free-ranging white rhinoceros immobilised with etorphine and azaperone corelated strongly and positively with adrenaline and noradrenaline concentration.[7] Adrenaline concentrations in the rhinoceros in this study were undetectable, so adrenaline cannot be the sole cause of the tremors. If noradrenaline were responsible, I would expect to observe a decrease in its plasma concentration over time as tremors dissipated, which I did not. There are several explanations that might account for this. My study may simply have been underpowered to detect a decrease in noradrenaline concentration over time. As mentioned above, it is possible that the level of etorphine-induced CNS depression was decreasing by t = 50 (slightly > 1 hour after darting) and rhinoceros were arousing; this could have caused sympathetic stimulation that increased noradrenaline concentration, masking a decrease that would explain the decrease in tremor score and confounding interpretation of the data. There is also some evidence that etorphine has antidopaminergic effects in the CNS that can produce tremor.[228] Thus, it is possible that noradrenaline is not the cause of the tremors, or at least not the *sole* cause. Although tremor intensity and catecholamine concentrations were strongly correlated in the publication by de Lange (2017), this does not prove that catecholamines *caused* the tremors.[7]

Comparisons with Variables in Chapter 2

In the study in Chapter 2, five white rhinoceros were darted IM with etorphine and azaperone, then administered butorphanol IV; a supplemental dose of etorphine was administered IV at 61 (53, 76) [median (minimum, maximum)] minutes after darting. Values for selected variables (mPAP, Qt, f_H, mSAP, PaO_2, and $PaCO_2$) were measured before (within 20 minutes) and after (within 10 minutes) this etorphine bolus. Values for the six variables

measured in Chapter 2 differed from values for these same variables measured between t = 30 and t = 50 in this study. Possible explanations for this discrepancy include 1) differences in when measurements were made relative to darting or 2) differences in the drugs administered. In this study, the mean time from darting to sternal recumbency (t = 0) was 12.4 ± 2.0 minutes; thus, variables at t = 30 were thus measured at ~40-45 minutes after darting whereas variables at t = 50 were thus measured ~60-65 minutes after darting. In Chapter 2, mPAP was 35 (32, 47) mm Hg (Figure 2-2A) at 40 (39, 75) minutes after darting. In this study, mPAP was 56-59 mm Hg from t = 30 to t = 50, broadly similar to when mPAP was measured in Chapter 2. This makes it less likely that mPAP differences between the two studies are temporal in origin. Examination of the raw data from Chapter 2 showed that a rhinoceros whose pre-etorphine mPAP was measured at 40 minutes and a rhinoceros whose pre-etorphine mPAP was measured at 75 minutes both had a mPAP in the low-to-mid 30s. It is therefore more likely that either the azaperone or butorphanol (or both) administered to the rhinoceros in Chapter 2 decreased mPAP through either vasorelaxation or decreased Qt. Although I was unable to locate any references that documented the effect of azaperone or butorphanol on PAP, azaperone is a known vasodilator.[67]

In Chapter 2, Qt was 120 (92, 145) L minute^{-1} (Figure 2-2B) at ~53 (47, 65) minutes after darting; normalized to body mass, this is 96 (84, 126) mL minute^{-1} kg^{-1}. In this study, Qt normalized to body mass was ~70-80 mL minute^{-1} kg^{-1} between t = 30 and t = 50. Thus, a longer period of time passed between darting and Qt measurement in Chapter 2 than in this study, and Qt was higher in Chapter 2. It is possible that the rhinoceros in Chapter 2 were recovering from the immobilization drugs and that Qt was higher due to sympathetic stimulation from CNS arousal. Another possibility is that the increased Qt was caused by vasodilation from the

administration of azaperone. As described in Chapter 1, a decrease in SAP decreases action potential generation in the baroreceptors and ultimately increases Qt.[108]

In Chapter 2, f_H before supplemental etorphine was 100 (88, 112) beats minute^{-1}, and mSAP before supplemental etorphine was 121 (86, 141) mm Hg (Figure 2-2C and D). These were measured 40 (39, 75) minutes after darting. In this study, f_H and mSAP measured between t = 30-50 was 116-121 beats minute^{-1} and 145-150 mm Hg, respectively. In other words, tachycardia and hypertension were worse in this study, possibly because f_H and mSAP were measured somewhat earlier in this study, when the sympathetic effects of etorphine were greater. However, examination of the raw data showed that f_H and time after darting did not appear to be correlated in Chapter 2. The same could be said about mSAP; mSAP possibly was higher in Chapter 3 because mSAP was measured somewhat earlier. A more likely explanation is that the lower f_H in Chapter 2 was due to the administration of butorphanol (Chapter 4), whereas the lower mSAP was due to the vasodilating effects of azaperone.[67]

In Chapter 2, before supplemental etorphine, PaO_2 and $PaCO_2$ measured at 42 (34, 52) minutes after darting was lower than PaO_2 and $PaCO_2$ measured from t = 30-50 in this study (35 mm Hg vs. ~30 mm Hg and 74 mm Hg vs. ~90 mm Hg, respectively, Figure 2-2E and F). Because the arterial blood gases in both studies were measured at broadly similar times after darting, it is most likely that the higher PaO_2 and lower $PaCO_2$ in Chapter 2 were caused by the administration of azaperone and butorphanol. In Chapter 2, pulmonary artery temperature was measured at the same times as blood gas partial pressures for the purpose of temperature correction. Pulmonary artery temperature before supplemental etorphine was 36.8 (35.8, 37.8)°C. This was lower than the mean pulmonary artery temperature of 38.2-38.4°C from t = 30-50 in this study. This could be due to the vasodilating effects of azaperone, which would facilitate heat

loss, or a decrease in metabolic rate, which would decrease heat production, caused by antagonism of the effects of etorphine by butorphanol (Chapter 4).[58]

Conclusions

The severe hypoxaemia observed in etorphine-immobilised rhinoceros is caused not only by hypoventilation but by venous admixture, which could result from either diffusion limitation, V:Q mismatching, shunting of blood past completely collapsed alveoli, or some combination of these factors. While V:Q mismatching due to atelectasis that develops secondary to recumbency likely contributes to some extent, my data support gas diffusion limitation as an important contributing factor. The increase in Qt that I observed could have caused gas diffusion limitation by decreasing erythrocyte transit time through pulmonary capillaries, or by increasing Pc and promoting the development of interstitial pulmonary oedema, both of which occur in maximally exercising horses and humans. An abnormally low $P\bar{v}O_2$ likely exacerbated the hypoxaemia and results from an increase in VO_2 that may be caused (at least in part) by skeletal muscle tremors. The haemodynamic changes and hypermetabolism are consistent with the high plasma noradrenaline concentrations measured in the rhinoceros. Remarkably, these data show that during immobilisation with etorphine, white rhinoceros, from a physiological standpoint, are actually galloping like racehorses. While this sympathetic upregulation might occur secondary to hypoxaemia and hypercapnia, direct stimulation of the SNS by etorphine is plausible. In summary, the pathophysiology behind the hypoxaemia observed in etorphine-immobilised rhinoceros is far more complex than wildlife veterinarians appreciate, and future research into preventative or therapeutic measures should focus not only on improving ventilation and supplementing oxygen but also on mitigating the sympathetic outflow induced by etorphine.

CHAPTER 4 INTRAVENOUS BUTORPHANOL IMPROVES MIXED VENOUS OXYGEN CONTENT IN ETORPHINE-IMMOBILISED WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*) PRIMARILY BY DECREASING OXYGEN CONSUMPTION RATHER THAN BY INCREASING OXYGEN DELIVERY

Abstract

Intravenous butorphanol increases arterial oxygen partial pressure (PaO_2) in etorphine-immobilised white rhinoceros, but its effect on oxygen delivery (DO_2) to tissues is unknown. I hypothesised that butorphanol would increase DO_2. To test this hypothesis, I administered two treatments to six sub-adult, male, white rhinoceros in random order, two weeks apart: etorphine-saline and etorphine-butorphanol. Rhinoceros were darted with etorphine (2.6 ± 0.1 µg kg^{-1} (mean ± standard deviation [SD]) intramuscularly by dart and positioned in sternal recumbency (time = 0 minutes [t = 0]). Rhinoceros were nasally intubated, connected to a custom breathing system with pneumotachometer, and positioned in left lateral recumbency. A peripheral arterial catheter and balloon-tipped thermodilution pulmonary arterial catheter were inserted. Baseline data were collected at t = 30, butorphanol (0.026 ± 0.001 mg kg^{-1}) or saline was administered intravenously at t = 37, and data were collected at t = 40 and 50. A linear mixed effect model was used to determine changes over time and between treatments. At t = 40 and 50, PaO_2 and arterial oxygen content (CaO_2) were greater after butorphanol than after saline (each $P < 0.0001$). Cardiac output (Qt) was lower after butorphanol at both times (each $P < 0.0001$). There was no difference in DO_2 between treatments. However, at both times, oxygen consumption (VO_2) was lower ($P = 0.0009$ and 0.0029, respectively) after butorphanol than after saline. Mixed venous oxygen partial pressure and content ($P\bar{v}O_2$ and $C\bar{v}O_2$) was greater (each $P < 0.0001$) after butorphanol at both times. Mixed venous lactate concentration was similar between treatments and was < 2 mmol L^{-1} in ~75% of samples. Despite the increase in CaO_2 after butorphanol, DO_2 to tissues did not increase uniformly because Qt decreased. However, the simultaneous decrease in VO_2 increased $P\bar{v}O_2$ and $C\bar{v}O_2$, indicating an improved balance between oxygen supply and demand.

Keywords: Butorphanol, cardiac output, etorphine, mixed venous oxygen content, oxygen delivery, white rhinoceros.

Introduction

The white rhinoceros (*Ceratotherium simum*) is poached heavily for its horn throughout southern Africa. Conservationists must manage white rhinoceros populations using strategies such as dehorning and translocation out of high-risk regions.[190] These procedures necessitate chemical immobilisation. Given the disposition and habitat of the white rhinoceros, chemical immobilisation can only be accomplished using a potent drug with a rapid onset of action that can be administered intramuscularly (IM) by dart and pharmacologically reversed. The ultra-potent μ opioid receptor agonist, etorphine, is one of only a few drugs that meet these criteria and is therefore indispensable for conservation of the species.

Unfortunately, chemical immobilisation with etorphine produces severe and life-threatening physiological perturbations in the white rhinoceros, including arterial hypoxaemia, hypercapnia, respiratory and metabolic (lactic) acidaemia, tachycardia, systemic arterial hypertension, tremors, and increased oxygen consumption (VO_2).[2-7; 17] Of these, hypoxaemia, an arterial oxygen partial pressure (PaO_2) < 90 mm Hg, is arguably the most significant.[44] These problems may cause unrecognised morbidity and occasionally result in mortalities during immobilisation.[1] Although they could be attributed to the flight response triggered by helicopter pursuit and darting in the field, these adverse effects have all been documented in white rhinoceros habituated to captivity and darted in bomas with etorphine only, demonstrating that etorphine is responsible, either directly or indirectly, for these perturbations.[2; 3]

Butorphanol is classified as an opioid receptor agonist-antagonist that acts as a full agonist at the κ opioid receptor but a partial agonist at the μ opioid receptor, which antagonises the μ opioid receptor-mediated effects of full μ opioid receptor agonists like etorphine.[31-34; 229] For this reason, it has been used to mitigate the adverse effects of etorphine in both boma and field settings for more than a decade.[61] Once a rhinoceros that has been darted with etorphine is deemed safe to approach, butorphanol is administered intravenously (IV) in an auricular vein immediately after contact with the rhinoceros is made. Butorphanol administration decreases heart rate (f_H), mean systemic arterial pressure (mSAP), arterial carbon dioxide partial pressure ($PaCO_2$), VO_2, and carbon dioxide production (VCO_2), and increases PaO_2 in boma-habituated white rhinoceros immobilised with etorphine alone.[2; 3] Although a μ opioid receptor antagonist such as naltrexone will reverse the μ opioid receptor-mediated adverse effects of etorphine, it will also reverse the central nervous system (CNS) effects (i.e., catatonia, analgesia) to an extent that would prohibit safe handling of the rhinoceros. Simultaneous κ opioid receptor agonism, which produces CNS depression, and partial μ opioid receptor agonism by butorphanol maintains an appropriate immobilisation level while mitigating hypoxaemia. Previous work has shown that decreased VO_2 and VCO_2 rather than increased minute ventilation (V_E) are primarily responsible for the improvement in PaO_2 after butorphanol administration.[2]

During immobilisation or anaesthesia, adequate tissue oxygenation is the foremost goal. While many factors impact how much oxygen reaches a cell's mitochondria, oxygen delivery (DO_2) is an important macrocirculatory value that must be maintained within normal limits. If oxygen supply (i.e., DO_2) cannot meet oxygen demand (i.e., VO_2) by tissues, VO_2 becomes supply-limited, oxygen debt develops, anaerobic metabolism increases, and lactic (metabolic) acidaemia ensues.[230] If oxygen debt is severe enough, cellular death occurs. Although the

beneficial effects of butorphanol on blood gases and VO_2 have been documented, a more comprehensive understanding of how butorphanol increases PaO_2, and if DO_2 and tissue oxygenation subsequently improve, requires pulmonary arterial catheterisation for measurement of pulmonary pressures, cardiac output (Qt), and mixed venous gas tensions and acid-base status.

My primary hypothesis was that butorphanol would increase DO_2 to tissues.

Materials and Methods

The University of Pretoria (UP) Animal Ethics Committee (AEC) (project number V101-15) and the South African National Parks (SANParks) Animal Use and Care Committee (AUCC, reference number 001/16) approved this research (Appendix).

Animals

Six sub-adult (4-5 years old), male, white rhinoceros were used in the experiments. The management and immobilisation of the rhinoceros followed the SANParks Standard Operating Procedures for the Capture, Transportation and Maintenance in Holding Facilities of Wildlife. The rhinoceros were captured via aerial darting in the Kruger National Park (KNP) (23°49’60 S, 31°30’0 E; altitude: 317 m above sea level), South Africa. Physical examination was performed to verify health. The rhinoceros were then group-housed in Veterinary Wildlife Services (VWS) bomas (i.e., wildlife holding enclosures, dimensions: 10.5 m * 21 m, or 220.5 m^2) where they were habituated to captivity over at least 1 month. Their diet consisted of lucerne (*Medicago sativa*) and tef (*Eragrostis tef*) hay and *ad libitum* water. Trained staff evaluated the rhinoceros daily using a scoring system developed by VWS based on the evaluation of: 1) feed intake, 2) volume, consistency, and colour of faeces, and 3) behaviour.[186]

During a pilot study, one rhinoceros suffered cardiac arrest during etorphine immobilisation and died, despite cardiopulmonary resuscitation efforts, including external chest

compressions, oxygen supplementation, and administration of naltrexone and epinephrine, by the investigators (including three board-certified anesthesiologists and four wildlife veterinarians). Because gross post-mortem examination did not reveal any pathology that could explain the arrest, the cause of death was deemed likely to be hypoxaemia. A second rhinoceros ingested a foreign object (a small wire), developed signs of esophageal obstruction ('choke') similar to horses. The rhinoceros was immobilised for examination, which revealed rupture and abscessation of the cervical esophagus, necessitating euthanasia. A third rhinoceros was excluded from the study because it scored '6' on the VWS immobilization scoring system (Table 4-3) during two immobilisation attempts and required urgent administration of the opioid antagonist, naltrexone, IV before data collection could be completed. This left a sample size of six rhinoceros for data collection and analysis.

Study Design

A controlled, randomised, crossover study design was used. Two treatments, etorphine-saline (ES) and etorphine-butorphanol (EB), were separated by a minimum 2 week washout period. *A priori*, rhinoceros were assigned to treatment order by random allocation in blocks of two by lottery, whereas an online randomiser (www.randomiser.org) determined the order of immobilisation of the rhinoceros. All experiments were conducted in the early morning. All drug doses were standard drug doses used by VWS based on estimated body mass (Table 4-1).

Table 4-1 Standard doses of drugs used in white rhinoceros by Veterinary Wildlife Services (VWS) based on body mass. All rhinoceros used in this study fell into one of these two categories.

Rhinoceros mass (kg)	Etorphine (mg)	Hyaluronidase (IU)	Butorphanol (10x etorphine dose, mg)	Naltrexone (20x etorphine dose, mg)
1,000 -1,250	2.5	5,000	25	50
1,250 – 1,500	3.125	5,000	31.25	62.5

IU, International Units

For both treatments, etorphine (M99; Voluplex, Mnandi, Centurion, South Africa) was delivered IM in the nuchal hump with a 3 mL plastic dart with a 60 mm uncollared needle fired from a compressed air rifle (DAN-INJECT International S.A., Skukuza, South Africa). As soon as a rhinoceros stopped moving, VWS staff experienced in the handling of white rhinoceros determined if it was safe to approach; if so, it was blindfolded and positioned in sternal recumbency using ropes. Sternal recumbency was designated as time = 0 minutes (t = 0) because it indicated a similar degree of CNS depression, reducing the effect of variation in induction times on the physiological variables to be measured. Instrumentation proceeded only if a rhinoceros could be positioned in sternal recumbency within 15 minutes of darting. Time between darting and sternal recumbency was recorded.

Thirty minutes was allotted for instrumentation once a rhinoceros had been positioned in sternal recumbency. Shortened equine endotracheal tubes (KRUSSE Silicone Endotracheal Tube, internal diameter: 28 mm, no. 282270; Jørgen Kruuse A/S, Langeskov, Denmark) were inserted into each naris in sternal recumbency, and the cuffs were inflated. The rhinoceros was

then positioned in left lateral recumbency, and instrumentation continued as described below. At t = 30, the first data set was collected. At t = 37, either butorphanol (Table 4-1, Butonil, Wildlife Pharmaceuticals Pty Ltd, White River, South Africa) or an equivalent volume of saline was administered IV in an auricular vein by the same investigator (PB). Data were collected again at t = 40 and 50. At the conclusion of the experiments, rhinoceros were de-instrumented, and butorphanol was administered IV to rhinoceros in treatment ES. Rhinoceros in both treatments were then stimulated to stand and walk in a controlled fashion into a crate for weighing. Naltrexone (Table 4-1, Kyron Laboratories Pty Ltd, Johannesburg, South Africa) was then administered IV, and the rhinoceros were released back into the bomas.

Instrumentation and Data Collection

Both of the equine endotracheal tubes in the nares were connected to an Exercise Physiology System with PowerLab 8/30 data acquisition hardware (ML870, ADInstruments Pty Ltd, New South Wales, Australia) and LabChart 7 data acquisition software with Metabolic Module (version 7.3.8, ADInstruments Pty Ltd) installed in a laptop computer used in a previously published white rhinoceros study (Figure 6-5).[2] Both endotracheal tubes were connected to Y pieces at the external margin of each naris. Each Y piece housed a one-way inspiratory valve, which allowed inspiration of ambient air, and a one-way expiratory valve (Two-Way Non-Rebreathing Valve, Series 2730; Hans Rudolph, Inc., Shawnee, Kansas, USA). Because the endotracheal tube cuffs were inflated and the mouth was closed, all expired gas was directed into a common T-piece, then a 4.7 L gas mixing chamber (MLA245; ADInstruments Pty Ltd). Expired gas temperature (in °C) was measured using a thermistor (Thermistor Temperature Sensor, MLT415/M, ADInstruments Pty Ltd) open to the gas-mixing chamber and connected to a temperature pod (ML309; ADInstruments Pty Ltd). Gas from the chamber was then directed

through a pneumotachometer (Respiratory Flow Head 1000L, MLT300L, ADInstruments Pty Ltd) and into the atmosphere, with the exception of a period of 1 minute at the beginning of each of the three sampling points, when a Douglas bag was attached to the pneumotachometer for collection of mixed expired gas. The pneumotachometer was capable of achieving laminar flow at flow rates up to 1000 L min^{-1} and was connected to a differential pressure transducer (Spirometer, ML141, ADInstruments Pty Ltd) for measurement of expired minute ventilation at body temperature and pressure, saturated with water vapour (V_{EBTPS}) from the pressure differential; before each immobilisation, the transducer was zeroed to atmospheric pressure and calibrated using a 3 L calibration syringe. Mixed expired carbon dioxide partial pressure ($P_{\bar{E}}CO_2$, mm Hg) and mixed expired oxygen fraction ($F_{\bar{E}}O_2$, %) were measured from the Douglas bag with the Cardiocap/5 (Datex-Ohmeda, GE Healthcare, Helsinki 00510, Finland), which auto-calibrated with ambient air on start-up and was also calibrated with a CO_2 canister. The Cardiocap/5 was also used to measure end-tidal oxygen and carbon dioxide partial pressures (P_EO_2 and $P_{ET}CO_2$, respectively) via a sampling port in one of the endotracheal tubes just outside the naris.

After aseptic skin preparation, a 22 standard wire gauge (SWG), 2.5 cm over-the-needle IV catheter (Nipro Medical Corporation, Bridgewater, New Jersey, USA) was inserted into a medial auricular artery. The catheter was connected by noncompliant tubing to a transducer (Deltran II pressure transducers, DPT-200, Utah Medical Products Inc., Midvale, Utah, USA) zeroed to atmospheric pressure at the *manubrium sterni*. The transducer was connected to a blood pressure amplifier (BP Amp, FE117, ADInstruments Pty Ltd), which was connected to the PowerLab 8/30 (ADInstruments Pty Ltd).

After aseptic skin preparation and draping of the angle of the mandible, a previously published technique was used to insert a percutaneous sheath introducer (11 Fr [3.67 mm], Super Arrow-Flex Sheath Set, Teleflex, Morrisville, North Carolina, USA) into the linguofacial branch of the right jugular vein under ultrasonographic guidance (Hitachi Noblus ultrasound system fitted with a C25 convex 5 – 1 MHz transducer, Hitachi Aloka Medical, Wallingford, Connecticut, USA).[17] A sterile, custom-built, 200 cm, 7 Fr (2.33 mm) Swan-Ganz-type thermodilution pulmonary arterial catheter (PAC, Gaeltec Devices, Dunvegan, Isle of Skye, Scotland) was then inserted through the introducer and down the jugular vein (Figure 6-1, Box 6-1). The distal port of the PAC was connected by noncompliant tubing to another Deltran II pressure transducer (Utah Medical Products) that was zeroed to atmospheric pressure at the level of the *manubrium sterni* and connected to the amplifier (ADInstruments Pty Ltd) and PowerLab 8/30 (ADInstruments Pty Ltd), which automatically performed a static calibration at 100 mm Hg. The balloon was inflated with a predetermined volume of air. This volume was determined just prior to each immobilisation by injecting air with a syringe into the pilot balloon until the moment the balloon inflated; the volume of air injected was recorded. Observation of the characteristic pressure waveforms on a laptop computer was used to pass the tip of the PAC into the pulmonary artery. Once a pulmonary arterial pressure (PAP) waveform was observed, the PAC was then advanced further until the balloon wedged in a branch of the pulmonary artery and a pulmonary arterial occlusion pressure (PAOP) waveform was observed on the monitor. A PAOP was recorded within 10 seconds of wedging, and the balloon was deflated. The PAC was withdrawn slightly and left with the tip in the pulmonary artery for subsequent pressure measurements. A thermistor at the tip measured pulmonary artery temperature (in °C).

Cardiac output was measured by thermodilution (PM-9000 Vet Veterinary Portable Multi-Parameter Patient Monitor, ShenZhen Mindray Bio-Medical Electronics Co., Nanshan, ShenZhen, China). The thermistor in the PAC had the same properties as the thermistor used in ARROW Balloon Thermodilution catheters (Teleflex). The appropriate computation constant (K in the Stewart-Hamilton equation) specified by Teleflex for that thermistor (0.308) and an injectate volume of 6 mL were entered into the monitor. Sixty mL (10 times the volume entered into the monitor) of ice-cold 0.9% saline was hand-injected by the same investigator (RG) within 3 seconds through the side port of the introducer into the linguofacial vein at end-expiration. Three to five sequential measurements were recorded at each sampling point and multiplied by a factor of 10 to obtain Qt in L minute^{-1}. The median value was used for analysis.

Arterial and mixed venous blood samples were collected simultaneously from the systemic arterial catheter and the distal port of the PAC, respectively. The arterial sample was analysed immediately with a point-of-care analyser (epoc Blood Analysis System, Siemens Medical Solutions, Inc., Malvern, Pennsylvania, USA) which produced results in less than 1 minute. The mixed venous sample was stored on ice and analysed immediately after the arterial sample. Values for pH and blood gas tensions were corrected to pulmonary artery temperature. A portion of each sample was spun down to obtain packed cell volume (PCV), which was used to calculate haemoglobin concentration ([Hb]) (Table 4-4, Figure 6-6). Blood was also collected from an auricular vein and added to ethylene-diamine-tetra-acetic acid (EDTA) tubes, which were immediately put on ice and spun down in a cold centrifuge at 2,500 revolutions minute^{-1} for 10 minutes. Plasma was pipetted into cryotubes (Greiner Bio-One, Frickenhausen, Baden-Württemberg, Germany), snap-frozen in liquid nitrogen, and stored at -80°C. This plasma was

used to measure catecholamine concentration with high-performance liquid chromatography-electrochemical detection (HPLC-ECD) using previously published methods (Box 6-8).[195]

Rectal temperature was measured using a thermometer inserted deep into the rectum against the rectal wall. Heart rate was measured via thoracic auscultation and confirmed using the SAP waveform. Severity of skeletal muscle tremors and immobilisation depth were scored by a single experienced, unblinded observer (PB) using scoring systems (Table 4-2, Table 4-3).

Table 4-2 Tremor scoring system for etorphine-immobilised white rhinoceros developed by Veterinary Wildlife Services (VWS).

Score	Description
1	No visible tremors
2	Mild tremors – resulting in minor leg and foot movement
3	Slight tremors – resulting in minor shoulder, chest and severe leg and foot movement
4	Moderate tremors – resulting in severe shoulder, chest, leg and foot movement
5	Severe tremors – resulting in whole body and head movement

Table 4-3 Immobilisation scoring system for etorphine-immobilised white rhinoceros developed by Veterinary Wildlife Services (VWS).

Score	Description
1	No effect
2	Cannot handle safely (standing)
3	Can handle safely (standing)

Table 4-3, continued

4	Recumbent, ear movements, tail curled
5	Recumbent, fully relaxed, no ear movements, tail relaxed
6	Recumbent, excessive depth, respiratory rate less than 3 breaths minute^{-1}

All data were collected by PowerLab 8/30 data acquisition hardware (ML870; ADInstruments Pty Ltd) and transmitted to a laptop computer with LabChart 7 data acquisition software (ADInstruments Pty Ltd). Values for mPAP, PAOP, and mean arterial pressure (mSAP) were measured and confirmed by analysing the pulmonary and systemic arterial pressure waveforms displayed by LabChart 7 (Box 6-2). Expired minute ventilation at body temperature and pressure, saturated with water vapour (V_{EBTPS}) and respiratory rate (f_R) were measured using the expiratory flow waveform displayed by LabChart 7 (Box 6-2). V_{EBTPS} was converted to expired minute ventilation, standard temperature and pressure, dry (V_{ESTPD}) (Table 4-4). Mixed expired carbon dioxide partial pressure was divided by P_B to calculate mixed venous carbon dioxide fraction ($F_{\bar{E}}CO_2$), which was used, along with $F_{\bar{E}}O_2$, to calculate oxygen consumption (VO_2, L minute^{-1}) (Box 6-7, Table 4-4). V_{ESTPD} was also used to calculate carbon dioxide production (VCO_2, L minute^{-1}) (Table 4-4). Pulmonary vascular resistance (PVR), stroke volume (SV), alveolar-arterial oxygen partial pressure difference (P_A-aO_2), arterial and mixed venous oxygen content (CaO_2 and $C\bar{v}O_2$), tidal volume (V_T), physiological dead space ventilation (V_{DPHYS}), VO_2, VCO_2, oxygen delivery (DO_2), oxygen extraction ratio (OER), pulmonary end-capillary oxygen content ($Cc'O_2$), and shunt fraction (Qs/Qt) were calculated using equations in **Error! Reference source not found.**.

Table 4-4 Equations for calculated variables in six boma-habituated, sub-adult, male white rhinoceros.

Calculated Variable	Equation
PVR (mmHg * minute L^{-1})	(mPAP – PAOP)/Qt
SV (mL $beat^{-1}$ kg^{-1})	Qt/f_H
P_AO_2 (mm Hg)	$F_IO_2 * (P_B - P_{WV}) – (PaCO_2/1)$
P_A-aO_2 (mm Hg)	$P_AO_2 – PaO_2$
[Hb] (g dL^{-1})	(0.2528 * PCV) + 4.013[a]
CaO_2 (mL dL^{-1})	([Hb] * 1.39 * SaO_2[a]) + (PaO_2 * 0.003)
$C\bar{v}O_2$ (mL dL^{-1})	([Hb] * 1.39 * $S\bar{v}O_2$[a]) + ($P\bar{v}O_2$ * 0.003)
$Cc'O_2$ (mL dL^{-1})	([Hb] * 1.39 * SO_2[b]) + (P_AO_2 * 0.003)
V_{ESTPD} (mL $minute^{-1}$ kg^{-1})	(273/310)[(P_B – 47)/760] * V_{EBTPS}
V_T (mL $breath^{-1}$ kg^{-1})	V_{ESTPD}/f_R
V_{DPHYS} (mL $minute^{-1}$ kg^{-1})	[($PaCO_2$ – $P_{\bar{E}}CO_2$)/$PaCO_2$]* V_T – 0.3[d]
DO_2 (mL $minute^{-1}$ kg^{-1})	CaO_2 * Qt
VO_2 (mL $minute^{-1}$ kg^{-1})	(V_I[c] * F_IO_2) - (V_E * F_EO_2)
VCO_2 (mL $minute^{-1}$ kg^{-1})	V_{ESTPD} * (F_ECO_2 – F_ICO_2)
OER (%)	VO_2/DO_2
Qs/Qt (%)	($Cc'O_2$ – CaO_2)/($Cc'O_2$ – $C\bar{v}O_2$)

PVR, pulmonary vascular resistance; mPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; Qt, cardiac output; SV, stroke volume; f_H, heart rate; P_AO_2, alveolar oxygen partial pressure; F_IO_2, inspired oxygen fraction; P_B, barometric pressure; P_{WV}, saturated water vapour pressure; $PaCO_2$, arterial carbon dioxide

Table 4-4, continued

partial pressure; $P_{A\text{-}a}O_2$, alveolar-arterial oxygen partial pressure difference; PaO_2, arterial oxygen partial pressure; [Hb], haemoglobin concentration; PCV, packed cell volume; CaO_2, arterial oxygen content; SaO_2, arterial oxyhaemoglobin saturation; $C\bar{v}O_2$, mixed venous oxygen content; $S\bar{v}O_2$, mixed venous oxyhaemoglobin saturation; $P\bar{v}O_2$, mixed venous oxygen partial pressure; $Cc'O_2$, pulmonary end-capillary oxygen content; SO_2, oxyhaemoglobin saturation (general); V_{EBTPS}, minute ventilation, body temperature and pressure standard; V_T, tidal volume; V_{ESTPD}, minute ventilation, standard temperature and pressure, dry; f_R, respiratory rate; V_{DPHYS}, physiological dead space ventilation; $P_{\bar{E}}CO_2$, mixed expired carbon dioxide partial pressure; DO_2, oxygen delivery; VO_2, oxygen consumption; V_I, inspired volume; V_E, minute ventilation or expired volume; F_EO_2, expired oxygen fraction; VCO_2, carbon dioxide production; F_ECO_2, expired carbon dioxide fraction; F_ICO_2, inspired carbon dioxide fraction; OER, oxygen extraction ratio; Qs/Qt, shunt fraction.

[a] Refer to Box 6-5 and Box 6-6 for further details.

[b] Values for $PaCO_2$ and arterial pH were used to calculate SO_2 in the formula for $Cc'O_2$.

[c] V_I was not necessarily equivalent to V_E because VO_2 did not necessarily equal VCO_2 (i.e., the respiratory exchange ratio [RER] was not necessarily 1). Therefore, the Haldane transformation was used to determine V_I.[196] Refer to Box 6-7 for further details.

[d] 0.3 L was subtracted to account for the volume of the tubes external to the nares.

Statistical Analysis

After performing a Shapiro-Wilk test to confirm normality of the data, a paired t-test was used to compare body mass of the rhinoceros, etorphine dose, time to sternal recumbency, and atmospheric temperature in the two treatments. Residuals were calculated, and the Shapiro-Wilk test was used to confirm that the residuals were normally distributed.

Statistical analysis was performed using commercial software (JMP 14.0.0, SAS Institute, Inc., Cary, North Carolina, USA). A linear mixed effect model was constructed, with each of the physiological variables designated as the response ('dependent') variables. Time, treatment (or 'phase'), and the interaction of time and treatment ('phase') were designated as fixed effects, and

rhinoceros identification number (ID) and rhinoceros ID nested within treatment were designated as random effects. *A priori*, mPAP, PAOP, Qt, PaO_2, mixed venous oxygen partial pressure ($P\bar{v}O_2$), DO_2, VO_2, and noradrenaline concentration were designated as the primary outcomes. The remaining variables were designated as secondary outcomes. Alpha was set at 0.05 for the eight primary outcomes, and 0.05/20 = 0.0025 for the 18 secondary outcomes (a Bonferroni correction for multiple comparisons). For each variable, the 36 residuals (three sampling points * two treatments * six rhinoceros) were calculated, and the Shapiro-Wilk test was used to confirm that the residuals were normally distributed. Residuals for PVR, SV, $P_A\text{-}aO_2$, f_R, noradrenaline concentration, and OER were not normally distributed; thus, the data for these variables was log-transformed, and residuals were re-tested to confirm normality.

Parameter estimates for all variables are listed in Chapter 6 (Table 6-2).

Results

In ES and EB, respectively, body mass of the rhinoceros was 1145 ± 73 kg and 1152 ± 98 kg, ambient temperature was 22.4 ± 2.2°C and 23 ± 3.8°C, etorphine dose administered was 2.6 ± 0.2 µg kg^{-1} and 2.6 ± 0.1 µg kg^{-1}, and time to sternal recumbency was 12.4 ± 2.0 minutes and 11.7 ± 2.0 minutes. There were no differences in any of these variables between ES and EB.

No complications arose from PAC insertion. The catheter was long enough to permit wedging in all rhinoceros in both ES and EB at all sampling points.

None of the variables were significantly different between ES and EB at t = 30 (baseline). Table 4-5 and Figure 4-1 depict differences within treatment EB over time, while Table 4-6 and Figure 4-1 depict differences between ES and EB at t = 30, 40, and 50. The data are depicted graphically in Figure 4-1, and parameter estimates generated by the linear mixed effects model are shown in Table 6-2. In summary, mPAP, PAOP, mSAP, Qt, f_H, pulmonary artery and rectal

temperatures, $PaCO_2$, V_T, OER, Qs/Qt, and tremor score in EB were significantly lower at one or more sampling points than contemporary values in ES (or were at least significantly lower than their baseline values at one or more sampling points) before Bonferroni correction. After butorphanol, PVR, PaO_2, $P\bar{v}O_2$, CaO_2, $C\bar{v}O_2$, V_{EBTPS}, f_R, and VCO_2 were significantly higher at one or more sampling points than contemporary values in ES (or were at least significantly higher than their baseline values at one or more sampling points). Some of the differences lost significance after Bonferroni correction, but their associated *P* values are listed because they demonstrate important trends that might have retained significance with a greater sample size or fewer comparisons. Stroke volume, $P(A\text{-}a)O_2$, V_{DPHYS}, and DO_2 in EB were not significantly different from ES at t = 40 or 50 and did not change significantly from t = 30 (baseline).

Table 4-5 Physiological variables in six boma-habituated, sub-adult, male white rhinoceros immobilised with etorphine intramuscularly (IM). After positioning in sternal recumbency (time = 0 minutes [t = 0]), data were collected at t = 30 (baseline), 40, and 50. Saline placebo or butorphanol was administered intravenously (IV) at t = 37. Blood gas partial pressures were corrected to pulmonary artery temperature. A linear mixed effect model was constructed to identify differences within treatments over time and between treatments at t = 30, 40, and 50. This table shows differences within EB over time. The primary outcomes are bold. Values in the sub-columns labeled '%' were calculated by dividing the value for each physiological variable at either t = 40 or t = 50 by the value for that physiological variable at t = 30. A value < 100% indicates the variable was lower at that time than at baseline, whereas a value > 100% indicates the variable was more at that time than at baseline. A *P* value of < 0.05 was considered significant (indicated by †) for the primary outcomes, whereas a *P* value of < 0.05/20 = 0.0025 (a Bonferroni correction for multiple comparisons) was considered significant for the secondary

Table 4-5, continued

outcomes. *P* values that lost significance after Bonferroni correction are listed but not indicated by †. NS = not significant.

	t = 40		t = 50	
	% of value at t = 30	***P***	**% of value at t = 30**	***P***
mPAP (mmHg)	77	0.0007†	73	0.0001†
PAOP (mmHg)	82	0.0031†	85	0.0145†
mSAP (mmHg)	79	0.0111	104	NS
Qt (mL minute^{-1} kg^{-1})	58	0.0004†	52	0.0001†
PVR (mmHg * minute L^{-1})	113	NS	114	NS
f_H (beats minute^{-1})	69	< 0.0001†	59	< 0.0001†
SV (mL beat^{-1} kg^{-1})	88	NS	89	NS
T (PA, °C)[a]	-	< 0.0001†	-	< 0.0001†
T (rectal, °C)[a]	-	NS	-	<0.0001†
PaO_2 (mmHg)	172	< 0.0001†	166	<0.0001†
$PaCO_2$ (mmHg)	72	< 0.0001†	76	<0.0001†
$P\bar{v}O_2$ (mmHg)	167	< 0.0001†	157	< 0.0001†
P_A-aO_2 (mmHg)	125	NS	112	NS
CaO_2 (mL dL^{-1})	186	< 0.0001†	178	< 0.0001†
$C\bar{v}O_2$ (mL dL^{-1})	260	< 0.0001†	237	< 0.0001†
V_{EBTPS} (mL minute^{-1} kg^{-1})	115	0.0293	99	NS

Table 4-5, continued

f_R (breaths minute^{-1})	183	0.0001†	167	0.0003†
V_T (mL breath^{-1} kg^{-1})	57	0.0089	54	0.0052
V_{DPHYS} (mL minute^{-1} kg^{-1})	110	NS	102	NS
VO_2 (mL minute^{-1} kg^{-1})	59	< 0.0001†	80	< 0.0001†
VCO_2 (mL minute^{-1} kg^{-1})	114	NS	95	0.0079
DO_2 (mL minute^{-1} kg^{-1})	115	NS	98	NS
OER (%)	51	0.0003†	81	NS
Qs/Qt (%)	50	< 0.0001†	49	< 0.0001†
Noradrenaline (ng mL^{-1})	92	NS	85	0.0004†
Tremor score	56	< 0.0001†	50	< 0.0001†
Lactate (mmol L^{-1})[b]	93	NS	80	NS
Hb (g dL^{-1})	98	NS	94	0.0003†

mPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; mSAP, mean systemic arterial pressure; Qt, cardiac output; PVR, pulmonary vascular resistance; f_H, heart rate; SV, stroke volume; T, temperature; PA, pulmonary artery; PaO_2, arterial oxygen partial pressure; $PaCO_2$, arterial carbon dioxide partial pressure; $P\bar{v}O_2$, mixed venous oxygen partial pressure; $P_{A}\text{-}aO_2$, alveolar-arterial oxygen partial pressure difference; CaO_2, arterial oxygen content; $C\bar{v}O_2$, mixed venous oxygen content; V_{EBTPS}, minute ventilation, body temperature and pressure, saturated with water vapour; f_R, respiratory rate; V_T, tidal volume; V_{DPHYS}, physiological dead space ventilation; VO_2, oxygen consumption; VCO_2, carbon dioxide production; DO_2, oxygen delivery; OER, oxygen extraction ratio; Qs/Qt, shunt fraction; Hb, haemoglobin.

[a] Because actual values rather than percent changes are typically listed when referring to temperature, the percent changes for T (PA and rectal) have not been included in this table, only the *P* values. Refer to Figure 4-1 for actual values for temperatures.

[b] Mixed venous lactate.

Table 4-6 Physiological variables in six boma-habituated, sub-adult, male white rhinoceros immobilised with etorphine intramuscularly (IM). After positioning in sternal recumbency (time = 0 minutes [t = 0]), data were collected at t = 30 (baseline), 40, and 50. Saline placebo or butorphanol was administered intravenously (IV) at t = 37. Blood gas partial pressures were corrected to pulmonary artery temperature. A linear mixed effect model was constructed to identify differences within treatments over time and between treatments at t = 30, 40, and 50. This table shows differences between ES and EB at t = 40 and 50; no variables were different between ES ad EB at baseline. The primary outcomes are bold. At each sampling point, '% change' was calculated as (value in EB – value in ES)/value in ES; thus, a negative value means that the physiological variable was X% lower in EB than in ES, and positive value means that the physiological variable was X% higher in EB than in ES. Alpha was set at 0.05 for the eight primary outcomes, and 0.05/20 = 0.0025 for the secondary outcomes (a Bonferroni correction for multiple comparisons). *P* values that lost significance after Bonferroni correction are listed but not indicated by †. NS = not significant.

	t = 40		**t = 50**	
	%	***P***	**%**	***P***
mPAP (mmHg)	-14%	NS	-19%	0.0153†
PAOP (mmHg)	-20%	0.0458†	-16%	NS
mSAP (mmHg)	-24%	0.0285	+1%	NS
Qt (mL minute^{-1} kg^{-1})	-43%	< 0.0001†	-44%	< 0.0001†
PVR (mmHg * minute L^{-1})	+58%	0.0371	+53%	NS
f_H (beats minute^{-1})	-30%	0.0071	-39%	0.0029

Table 4-6, continued

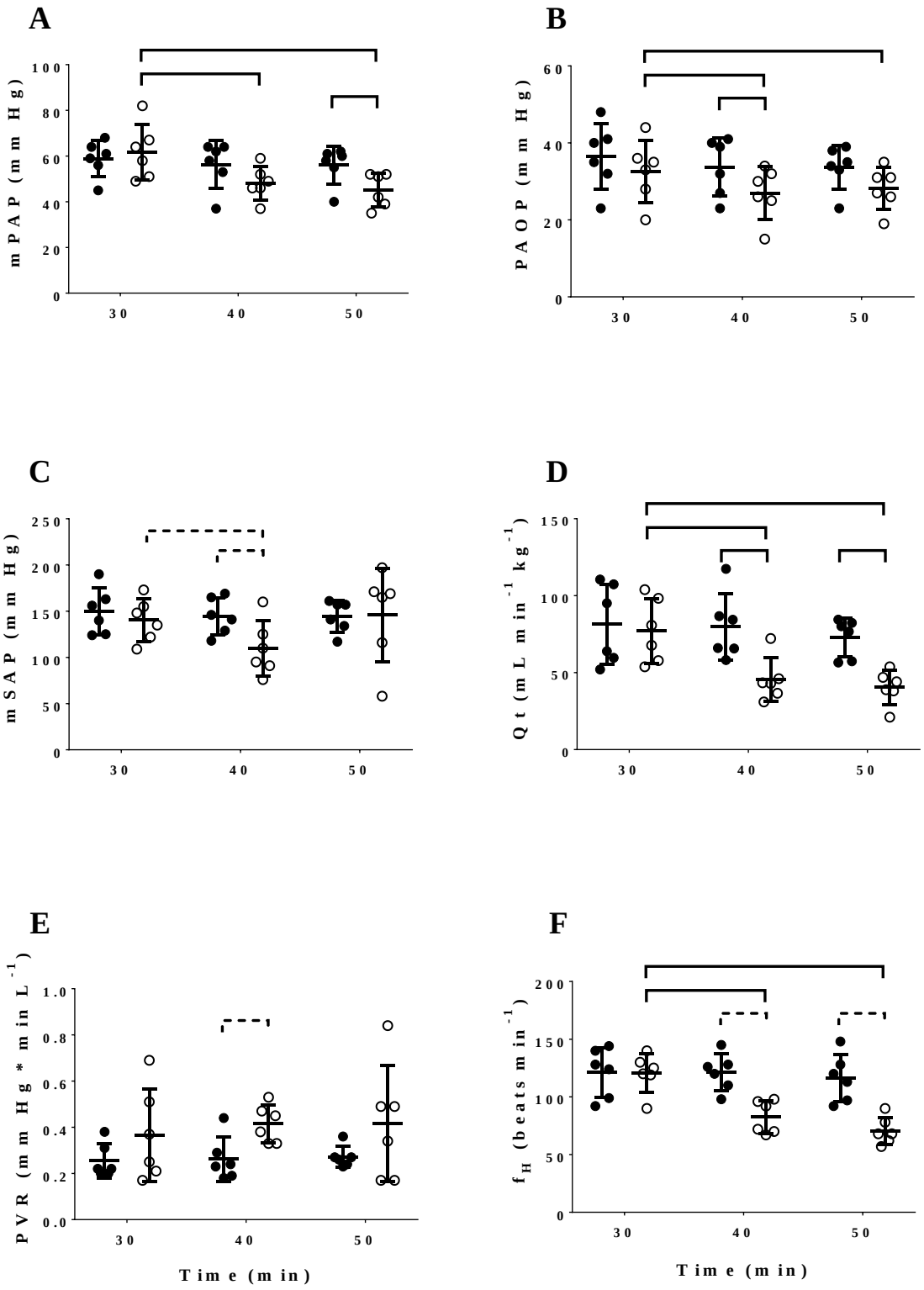
SV (mL beat^{-1} kg^{-1})	-12%	NS	-6%	NS
T (PA, °C)[a]	-	0.0030	-	0.0107
T (rectal, °C)[a]	-	NS	-	0.0054
PaO_2 (mmHg)	+63%	< 0.0001†	+53%	< 0.0001†
$PaCO_2$ (mmHg)	-29%	0.0005†	-20%	0.0059
$P\bar{v}O_2$ (mmHg)	+61%	< 0.0001†	+40%	< 0.0001†
P_{A}-aO_2 (mmHg)	+40%	NS	-26%	NS
CaO_2 (mL dL^{-1})	+81%	< 0.0001†	+56%	< 0.0001†
$C\bar{v}O_2$ (mL dL^{-1})	+161%	< 0.0001†	+103%	< 0.0001†
V_{EBTPS} (mL minute^{-1} kg^{-1})	+17%	0.0418	+5%	NS
f_R (breaths minute^{-1})	+73%	0.0012†	+35%	0.0427
V_T (mL breath^{-1} kg^{-1})	-39%	0.0356	-31%	NS
V_{DPHYS} (mL minute^{-1} kg^{-1})	+4%	NS	-4%	NS
VO_2 (mL minute^{-1} kg^{-1})	-36%	0.0009†	-28%	0.0029†
VCO_2 (mL minute^{-1} kg^{-1})	+18%	NS	-15%	NS
DO_2 (mL minute^{-1} kg^{-1})	+6%	NS	-10%	NS
OER (%)	-43%	0.01	-18%	NS
Qs/Qt (%)	-46%	< 0.0001†	-51%	< 0.0001†
Noradrenaline (ng mL^{-1})	-1%	NS	-2%	NS
Tremor score	25%	NS	-34%	0.0217
Lactate (mmol L^{-1})[b]	-12%	NS	-13%	NS
Hb (g dL^{-1})	+4%	NS	-1%	NS

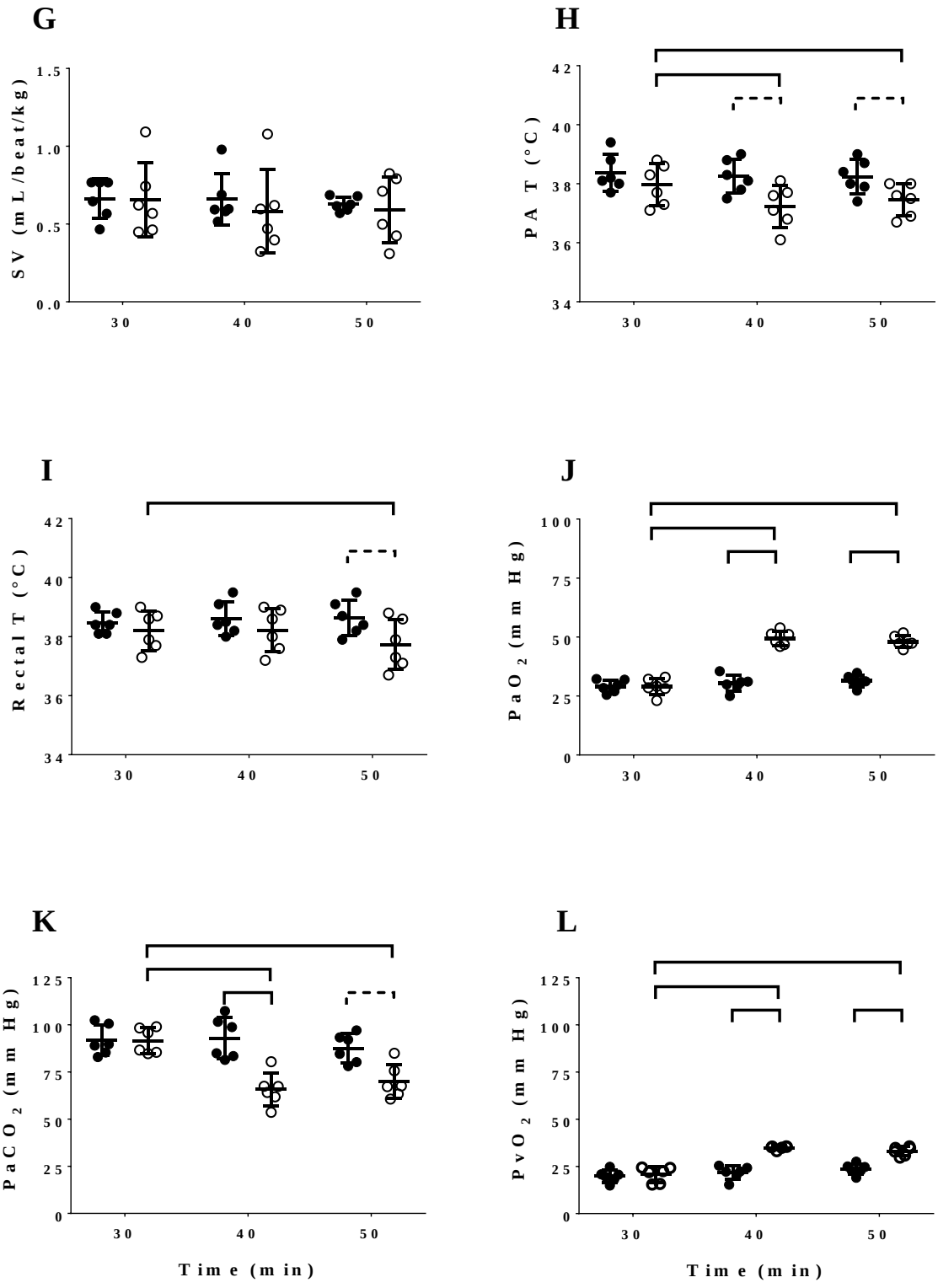
Table 4-6, continued

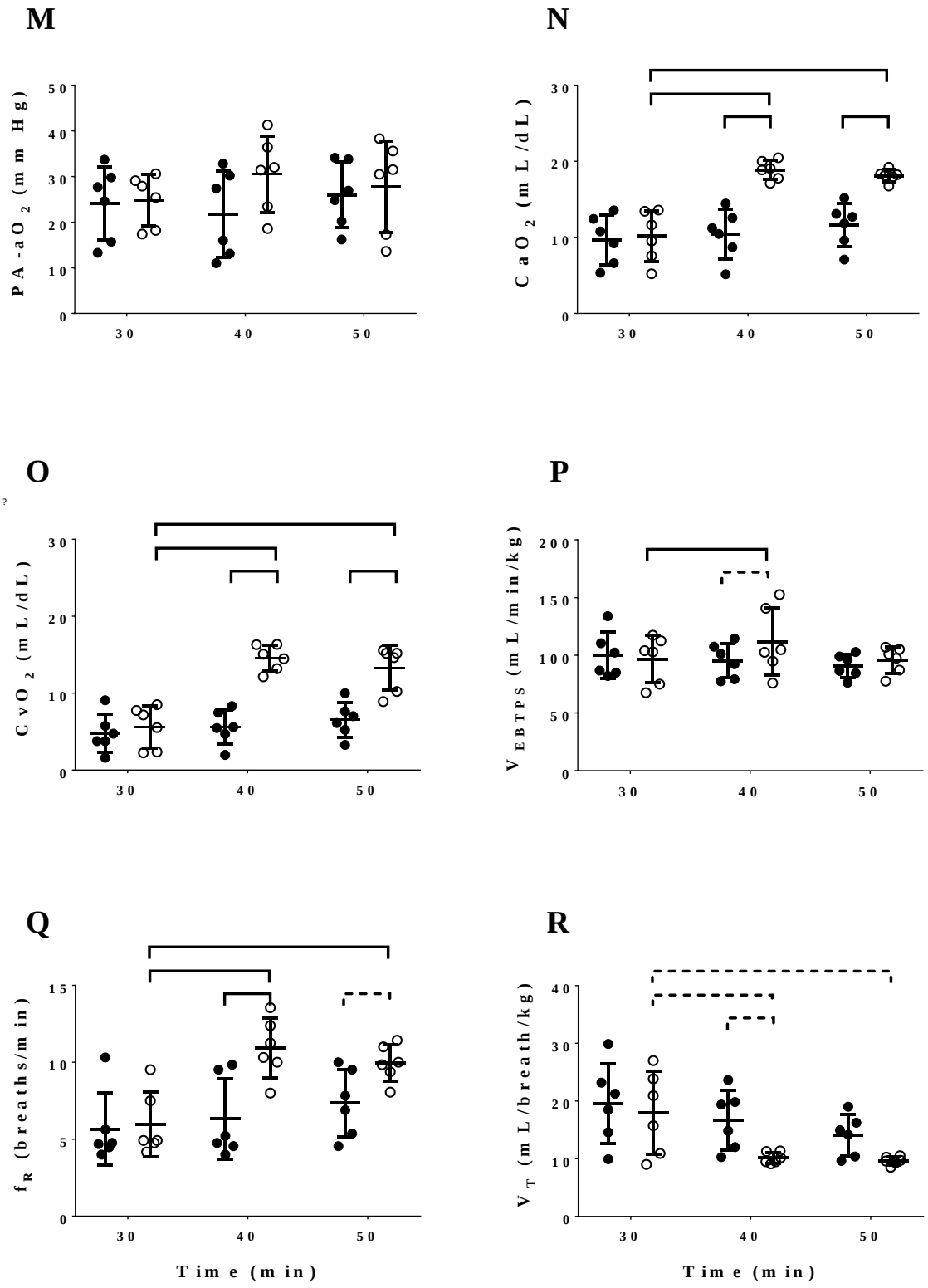
mPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; mSAP, mean systemic arterial pressure; Qt, cardiac output; PVR, pulmonary vascular resistance; f_H, heart rate; SV, stroke volume; T, temperature; PA, pulmonary artery; PaO_2, arterial oxygen partial pressure; $PaCO_2$, arterial carbon dioxide partial pressure; $P\bar{v}O_2$, mixed venous oxygen partial pressure; $P_{A}\text{-}aO_2$, alveolar-arterial oxygen partial pressure difference; CaO_2, arterial oxygen content; $C\bar{v}O_2$, mixed venous oxygen content; V_{EBTPS}, minute ventilation, body temperature and pressure, saturated with water vapour; f_R, respiratory rate; V_T, tidal volume; V_{DPHYS}, physiological dead space ventilation; VO_2, oxygen consumption; VCO_2, carbon dioxide production; DO_2, oxygen delivery; OER, oxygen extraction ratio; Qs/Qt, shunt fraction; Hb, haemoglobin.

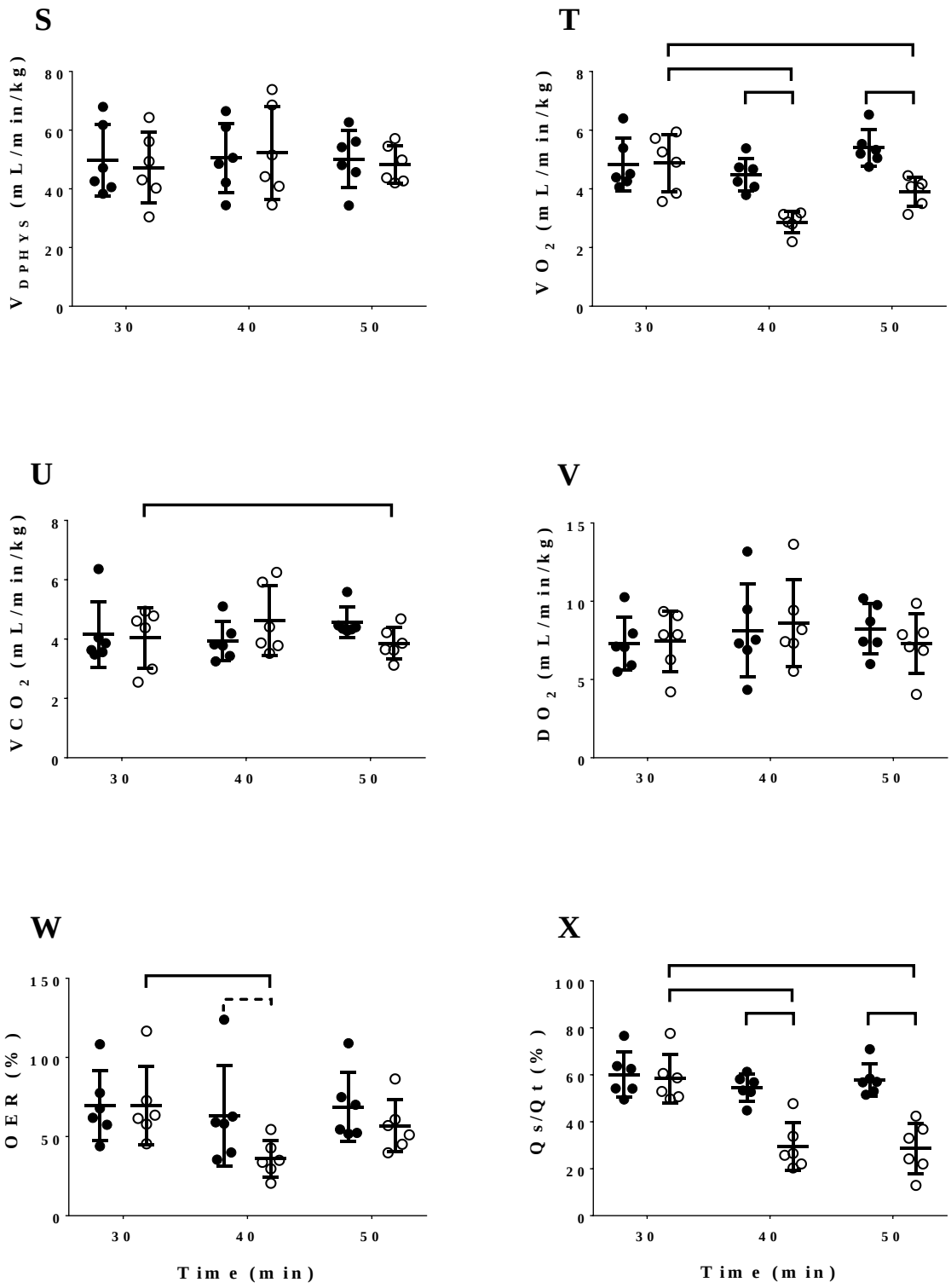
[a] Because actual values rather than percent changes are typically listed when referring to temperature, the percent changes for T (PA and rectal) have not been included in this table, only the *P* values. Refer to Figure 4-1 for actual values for temperatures.

[b] Mixed venous lactate.


A
mPAP (mm Hg)
100
80
60
40
20
0
30
40
50
B
PAOP (mm Hg)
60
40
20
0
30
40
50
C
mSAP (mm Hg)
250
200
150
100
50
0
30
40
50
D
Qt (mL min-1 kg-1)
150
100
50
0
30
40
50
E
PVR (mm Hg * min L-1)
1.0
0.8
0.6
0.4
0.2
0.0
30
40
50
Time (min)
F
fH (beats min-1)
200
150
100
50
0
30
40
50
Time (min)


G
SV (mL/beat/kg)
1.5
1.0
0.5
0.0
30
40
50
H
PAT (°C)
42
40
38
36
34
30
40
50
I
Rectal T (°C)
42
40
38
36
34
30
40
50
J
PaO2 (mm Hg)
100
75
50
25
0
30
40
50
K
PaCO2 (mm Hg)
125
100
75
50
25
0
30
40
50
Time (min)
L
PvO2 (mm Hg)
125
100
75
50
25
0
30
40
50
Time (min)


M
N
O
P
Q
R
PA-aO2 (mm Hg)
CaO2 (mL/dL)
CvO2 (mL/dL)
VEBTPS (mL/min/kg)
fR (breaths/min)
VT (mL/breath/kg)
Time (min)
Time (min)


S
T
U
V
W
X
V_{DPHYS} (mL/min/kg)
VO_2 (mL/min/kg)
VCO_2 (mL/min/kg)
DO_2 (mL/min/kg)
OER (%)
Qs/Qt (%)
30
40
50
Time (min)

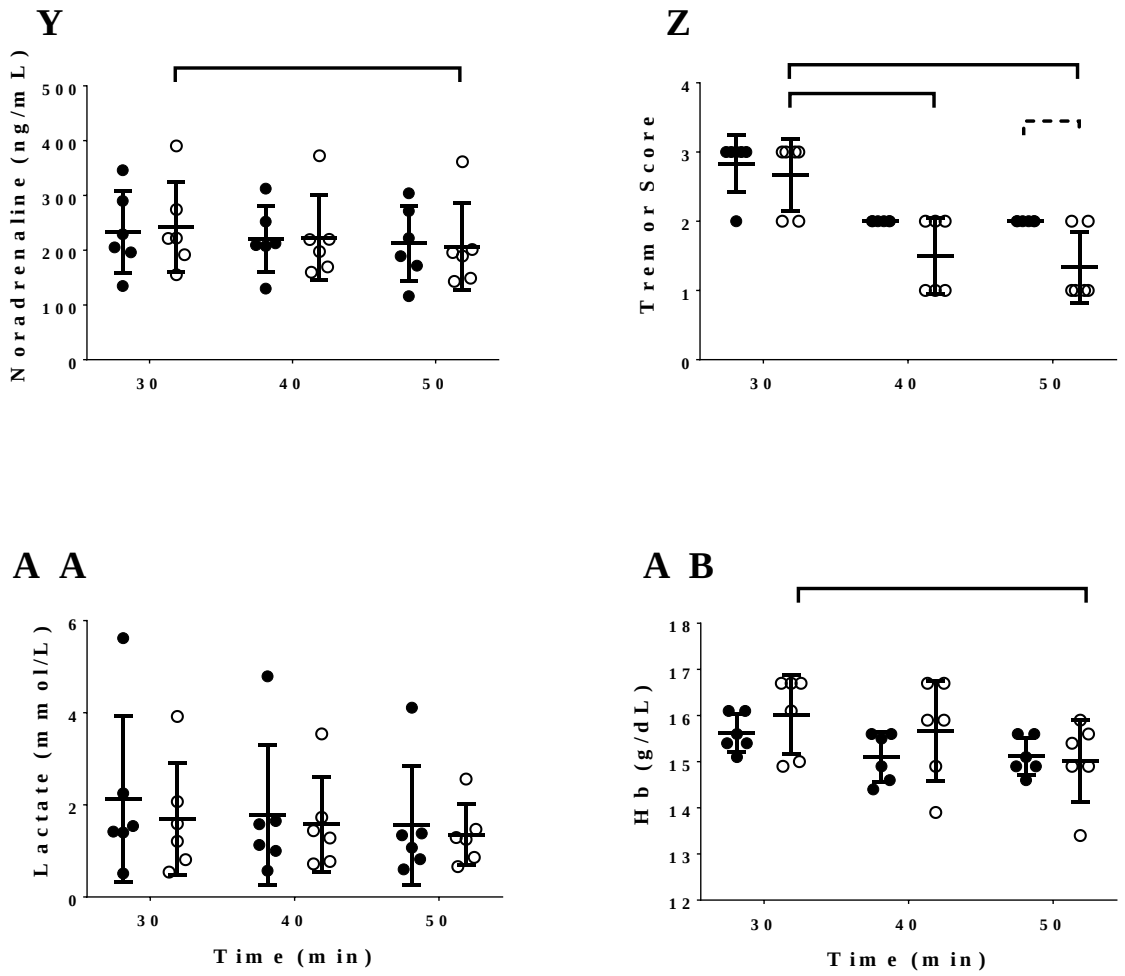


Figure 4-1A-AB Graphs of each variable measured in six boma-habituated, sub-adult, male white rhinoceros. Closed circles, etorphine-saline (ES); open circles, etorphine-butorphanol (EB). The middle bar in each data set represents the mean, whereas the top and bottom bars represent the standard deviation (SD). A linear mixed effect model was constructed to evaluate differences between treatments, and within treatment EB over time. The data sets connected by solid bars are significantly different; those connected by dashed bars were no longer significantly different after Bonferroni correction.

mPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; mSAP, mean systemic arterial pressure; Qt, cardiac output; PVR, pulmonary vascular resistance; f_H, heart rate; SV, stroke volume; PA, pulmonary artery; T, temperature; PaO_2, arterial oxygen partial pressure; $PaCO_2$, arterial carbon dioxide partial pressure; $P\bar{v}O_2$, mixed venous oxygen partial pressure; $P_{A}\text{-}aO_2$, alveolar-arterial oxygen partial pressure difference; CaO_2, arterial oxygen content; $C\bar{v}O_2$, mixed venous oxygen content; V_{EBTPS}, minute ventilation, body temperature and pressure, saturated with water vapour; f_R, respiratory rate; V_T, tidal volume; V_{DPHYS}, physiological dead space ventilation; VO_2, oxygen consumption; VCO_2, carbon dioxide production; DO_2, oxygen delivery; OER, oxygen extraction ratio; Qs/Qt, shunt fraction; Hb, haemoglobin.

Discussion

An important limitation of this research is the small sample size. Considering the size of white rhinoceros, and the expense and logistics associated with capturing and maintaining them in captivity for an extended time period, a larger sample size was not possible. Furthermore, because of the great intrinsic value of each rhinoceros, and the inherent risk associated with immobilisation, the smallest sample size that was likely to demonstrate significant differences between treatments was used. Thus, it is not surprising that the study was underpowered to detect differences between treatments for some outcomes. Nevertheless, I was able to detect differences between treatments that allowed me to develop a picture of the effects of butorphanol.

Since the advent of arterial blood gas analysis in etorphine-immobilised white rhinoceros, when perilously low PaO_2 values were first observed, veterinarians have sought ways, including the administration of butorphanol, to increase PaO_2, assuming that an increase in PaO_2 would improve CaO_2, and thus, DO_2 and tissue oxygenation. Consistent with previous work, butorphanol did produce a significant improvement in PaO_2, from ~30 mm Hg at baseline to ~45-55 mm Hg at t = 40 and t = 50.[2; 6] However, PaO_2 is ~90-100 mm Hg in conscious, resting,

standing, unmedicated white rhinoceros, as in horses, thus, none of the rhinoceros achieved normoxaemia.[44; 171]

Despite the persistent hypoxaemia, the increase in PaO_2 was sufficient to increase CaO_2 from ~10 mL dL^{-1} (approximately half the normal value documented in horses, or ~20 mL dL^{-1}) at baseline (t = 30) to near-normal values (19 mL dL^{-1} and 18 mL dL^{-1}) at t = 40 and t = 50.[105] Given that [Hb] did not increase significantly after butorphanol, this increase in CaO_2 is likely predominantly influenced by the oxyhaemoglobin dissociation curve (ODC) of the white rhinoceros. The white rhinoceros' P_{50}, the oxygen partial pressure (PO_2) at which haemoglobin (Hb) is 50% saturated, has been determined *ex vivo* to be 2.3 kPa (17.4 mm Hg, when [Hb] = 4 g dL^{-1}, T = 37°C, pH = 7.2, and PCO_2 = 40 mm Hg).[231] A recent publication showed that the P_{50} (at pH = 7.2) is 2.75 ± 0.07 kPa (20.6 mm Hg), closer to the value used by Haymerle et al. (2016) that I also used in my calculations (Box 6-6).[232; 233] This value is lower than the P_{50} of dogs (3.8 kPa or 28.8 mm Hg), humans (3.5 kPa or 26.6 mm Hg) and horses (3.2 kPa or 23.8), which indicates that the haemoglobin of the white rhinoceros has a relatively higher intrinsic oxygen affinity than most species and thus requires a lower PO_2 to 50% saturate its haemoglobin.[234; 235] Substitution of a glutamic acid for a glutamine residue at the β_2 position of haemoglobin appears to impart this characteristic.[236] Thus, the values for SaO_2 (and $S\bar{v}O_2$) that were required to calculate CaO_2 (and $C\bar{v}O_2$) were derived in the most accurate way possible to date.[237; 238] Calculating rhinoceros-specific SaO_2 (and $S\bar{v}O_2$) values showed that the values for SaO_2 (and $S\bar{v}O_2$) given by the epoc Blood Analysis System, which are calculated based on the human ODC, were, unsurprisingly, lower. Although a minor degree of inaccuracy in my calculations is likely, it seems reasonable to conclude that the improvement in PaO_2 after

butorphanol administration was sufficient to reach the steep middle portion of the sigmoidal ODC, resulting in a sharp increase in SaO_2.

Several mechanisms might account for the improvement in PaO_2 and CaO_2 after butorphanol administration. Mu opioid receptors in various regions of the brainstem mediate hypoventilation.[239] Antagonism improves central respiratory drive, increasing V_{EBTPS}; V_{EBTPS} was 17% higher ($P = 0.0418$) at t = 40 in treatment EB compared to treatment ES and had increased from baseline by 16% within treatment EB ($P = 0.0293$), although these differences lost significance after Bonferroni correction. However, consistent with the findings of Buss et al. (2018), this effect was, unfortunately, only transient; by t = 50, V_{EBTPS} was no longer significantly higher in treatment EB or significantly higher than baseline within treatment EB.[2] The increase in V_{EBTPS} was due to an increase in f_R rather than V_T, which actually decreased, again consistent with the findings of Buss et al. (2018).[2] The transient improvement in V_E (at least in part) decreased $PaCO_2$. A decrease in $PaCO_2$ (and thus, alveolar carbon dioxide partial pressure) decreases displacement of alveolar oxygen and increases P_AO_2; in the absence of other causes of hypoxaemia (gas diffusion limitation, ventilation:perfusion [V:Q] mismatching, shunt), this will increase PaO_2.[240] An improvement in V_E can also improve V:Q mismatching indirectly by expanding collapsed alveoli, decreasing atelectasis (below). However, given that V_T decreased and $P_A\text{-}aO_2$ did not change, it seems unlikely that decreased atelectasis played a role.

Butorphanol may have improved hypoxaemia by decreasing gas diffusion limitation.[198] Diffusion limitation can occur if 1) erythrocyte transit time through pulmonary capillaries is decreased or 2) interstitial pulmonary oedema develops.[198] If the flow of blood (right-sided Qt) through pulmonary capillaries is too great, the time available for oxygen to diffuse across the alveolar-capillary membrane and bind to haemoglobin is insufficient and results in abnormally

low $Cc'O_2$.[198] Diffusion limitation is a major mechanism responsible for hypoxaemia in the exercising horse and is caused by tachycardia and thus, increased Qt.[113; 116; 168; 178] Heart rate in standing, unrestrained white rhinoceros is 32-42 beats $minute^{-1}$, whereas mean f_H in these rhinoceros was ~120 beats $minute^{-1}$ at t = 30.[44] Heart rate was lower in treatment EB than in treatment ES at t = 40 and t = 50, similar to the findings of Buss et al. (2015).[3] Although changes in f_H between treatments lost significance after Bonferroni correction, f_H did decrease significantly over time in EB. Butorphanol did not change SV significantly, demonstrating that the decrease in f_H is responsible for the substantial decrease in Qt observed in the rhinoceros, as in the horse. The decrease in Qt would increase erythrocyte transit time and thus, time for oxygen equilibration. The decrease in f_H and Qt would explain the decrease in mPAP, PAOP, and mSAP (although the difference in mSAP between treatments at t = 40 and the difference in mSAP between t = 30 and 40 lost significance after Bonferroni correction). The decrease in Qt would also explain the increase in PVR at t = 40 (although this lost significance after Bonferroni correction). The increase in PVR could be caused by an increase in the difference between mPAP and PAOP (e.g., due to vasoconstriction and increased mPAP, or decreased PAOP, or both), but I did not observe an increase in this difference.

Diffusion limitation can also occur when interstitial pulmonary oedema develops and plays an important role in the hypoxaemia observed in exercising horses and humans.[168; 198] An increase in f_H and Qt increases pulmonary capillary pressure (Pc), creating an imbalance in Starling's forces (Chapter 1), which increases the rate of extravasation of fluid from the capillary lumen into the interstitium.[168] If lymphatic drainage is insufficient to accommodate this increased flow, interstitial pulmonary oedema develops (Chapter 1). Although estimation of Pc, which has been performed in the horse, was not possible in this study, Pc must be somewhere

between mPAP and PAOP.[168] At baseline, mPAP was ~60 mm Hg, and PAOP was ~35 mm Hg. Pc between 35-60 mm Hg is sufficient to cause accumulation of extravascular lung water in sheep.[241] A PAOP of 20-25 mm Hg is generally associated with the development of interstitial oedema in humans.[177] Thus, these rhinoceros achieved Pc known to cause pulmonary oedema in other species. By decreasing Pc, butorphanol may restore the balance of Starling's forces, reducing the burden on the pulmonary lymphatics and resolving interstitial oedema.[175] Butorphanol could decrease Pc by decreasing f_H (above) which would 1) decrease right-sided Qt and 2) give the left ventricle more time to relax, decreasing left ventricular end-diastolic pressure (LVEDP).[174] Because the pressure in the pulmonary veins, left atrium, and left ventricle equilibrate during diastole, decreased LVEDP ultimately results in lower Pc. How butorphanol decreases f_H is unknown and could not be determined by this study, but it could be a result of the direct effects of butorphanol (by its effects at the μ opioid receptor, or some other mechanism, such as vagal stimulation) or indirectly (by improving $PaCO_2$ and PaO_2 and decreasing the sympathetic outflow that occurs in response to hypercapnia and hypoxaemia).[226]

Butorphanol may increase PaO_2 by reducing V:Q mismatching. In these rhinoceros, a reduction in V:Q mismatching might occur through resolution of atelectasis or alveolar pulmonary oedema. The latter can occur when the interstitium can no longer contain the increased fluid flux from pulmonary capillaries and floods the alveoli.[177] An improvement in central respiratory drive could increase diaphragmatic contraction and thoracic expansion, which could open collapsed alveoli and improve the PAO_2 of alveolar units with low V:Q. However, if alveolar expansion had occurred, V_T should have increased when in fact, consistent with previous work, it decreased.[2] Alveolar pulmonary oedema would occur if Starling's forces (above) were sufficiently unbalanced by the increase in Qt and Pc to cause a flow of fluid from

capillary to interstitium great enough to overwhelm the capacity of the interstitium, resulting in alveolar flooding.[177] This flooding may explain reports from wildlife veterinarians of foam, sometimes sanguinous, at the nares of rhinoceros that have died during immobilisation (Prof. Leith Meyer, personal communication). Butorphanol might improve the balance of Starling's forces by decreasing f_H and Qt, thus decreasing Pc and permitting the resolution of pulmonary oedema. When alveoli are completely collapsed (V:Q = 0), shunting of blood occurs. Butorphanol improved Qs/Qt which, again, might have been through resolution of atelectasis or absorption of oedema.

One would expect that a butorphanol-induced improvement in diffusion limitation and V:Q mismatching would decrease P_A-aO_2, but consistent with previous work, P_A-aO_2 did not change significantly.[2] There are a number of possibilities that might explain this finding. Once alveoli are collapsed, high pressures are typically required to recruit (open) them. If interstitial or alveolar oedema ocurred, it may take time for it to resolve; oedema after exercise in humans has been shown to persist for 12 hours.[200] One scenario that supports the latter explanation is a butorphanol-induced improvement in ventilation and gas diffusion limitation balanced by increased V:Q mismatching due to progression of atelectasis. However, Qs/Qt decreased significantly after butorphanol. A decrease of this magnitude would be expected to decrease P_A-aO_2. In any case, Qs/Qt is a more accurate calculation of pulmonary oxygen transfer than P_A-aO_2, one of the so-called "oxygen indices", in critically ill people.[242] In patients with a changing respiratory quotient (RQ), which the rhinoceros almost certainly were, the alveolar gas equation may not produce accurate results.[243] Shunt fraction is discussed further below.

Although decreased $P\bar{v}O_2$ (and thus, $S\bar{v}O_2$ and $C\bar{v}O_2$) does not cause hypoxaemia, it can exacerbate a decrease in PaO_2 produced by other mechanisms. At baseline (t = 30) in both

treatments, $P\bar{v}O_2$ was ~20 mm Hg, half the $P\bar{v}O_2$ in standing horses and other mammals (~40 mm Hg).[105] At t = 40 and t = 50, $P\bar{v}O_2$ in treatment EB had increased to 33-35 mm Hg. Increased $P\bar{v}O_2$ and $C\bar{v}O_2$ is caused by either increased DO_2 or decreased VO_2 and, along with lactate concentration, is an important indicator of global oxygen-supply balance. Because DO_2 was unchanged by butorphanol, the increase in $P\bar{v}O_2$ must primarily reflect the decrease in VO_2, which was about a third lower in treatment EB than in treatment ES at t = 40 and t = 50. Tremor score was significantly lower in treatment EB than in treatment ES at t = 50; the difference approached, but did not achieve, significance at t = 40. This finding is consistent with decreased VO_2, as well as the decrease in OER I observed. Muscle contraction, as during exercise, shivering, seizures, or hyperthermia, consumes a substantial volume of oxygen, increasing metabolic rate in the muscle.[105; 244] Catecholamine agonism of β_2 adrenoceptors in skeletal muscles may be responsible for the tremors; etorphine may also exert anti-dopaminergic effects that could cause tremors.[228; 245] Butorphanol may mitigate tremors either directly or indirectly (by mitigating hypoxaemia and hypercapnia, as discussed above).[226] Even if tremors were not observed, sympathoadrenal outflow could have caused a generalised increase in VO_2 due to increased metabolism in other tissues, as it does in other resting mammals.[244; 246] Migitation of tremors or reduction in overall metabolic rate by butorphanol could also explain the lower rectal temperature in EB, which was closer to the mean value of 36.8°C reported by Citino and Bush (2007) (Table 1-1), than in ES at t = 50 and the lower pulmonary artery temperature in EB than in ES at t = 40 and 50, as discussed in Chapter 3; a decrease in body temperature could have contributed to changes in other physiological variables, such as the decrease in f_H.[218; 220; 222] The decrease in VO_2 could also explain the decrease in the oxygen extraction ratio (OER) in these rhinoceros.[214; 215]

Shunt fraction is ideally calculated while a patient is breathing 100% oxygen, which will minimise or eliminate the effects of diffusion limitation and V:Q mismatching, leaving only the effects of anatomical or physiological shunting.[247] However, because these rhinoceros were breathing ambient air, the Qs/Qt values I observed encompass diffusion limitation and V:Q mismatching as well.[247] Furthermore, $C\bar{v}O_2$ can affect the Qs/Qt calculation, so it is possible that the decrease I observed was caused by the increase in $C\bar{v}O_2$.[247]

The only change in noradrenaline concentration was at t = 50, when it was lower than its baseline value within EB. Tremor score was lower at t = 40 and 50 compared to baseline within EB, as it was within treatment ES (Chapter 3). The only difference between ES and EB occurred at t = 40, when tremor score was lower in EB. This lost significance after Bonferroni correction but points to the possibility that butorphanol somehow decreases noradrenaline concentration, either directly (e.g., by antagonism of the μ opioid receptor) or indirectly (e.g., by mitigating hypoxaemia and hypercapnia). As mentioned in Chapter 3, the butorphanol may have decreased the depth of immobilisation, which might have increased sensory stimulation and thus, noradrenaline concentrations, confounding interpretation of the results. A more dramatic difference might have been detected with a greater sample size.

Oxygen delivery is the product of CaO_2 and Qt. As discussed above, butorphanol caused a dramatic decrease in mean Qt in all rhinoceros by decreasing f_H. This decrease balanced the increase in CaO_2, resulting in unchanged DO_2. As described above and in Chapter 1, an imbalance of oxygen supply and demand will result in anaerobic metabolism and lactic (metabolic) acidaemia. Lactate concentration in mixed venous blood (blood in the pulmonary artery, where blood returning from all body tissues combines and mixes) reflects global tissue oxygenation.[248; 249] Mixed venous lactate was relatively low (< 2 mmol L^{-1}) in most rhinoceros

(with some higher values) when compared with, for instance, rhinoceros darted in the field, or endotoxaemic or exercising horses, and was unaffected by butorphanol.[5; 250; 251] However, it was slightly increased compared to resting horses, in which lactate concentration is ~0.5 mmol L^{-1}, so the values I observed may be increased above normal; resting lactate concentration has not been measured in conscious white rhinoceros.[44; 252] In the horse, the elimination half-life of lactate, which is metabolised primarily by the liver, is ~30 minutes.[252] Butorphanol was administered at t = 37, allowing only 3 (t = 40) and 13 minutes (t = 50) for it to exert an effect on lactate concentration; it was unlikely that sufficient time had passed to allow detection of a change after butorphanol. However, the improvement in $C\bar{v}O_2$ shows that, despite lactate concentrations that were not severely increased, the administration of butorphanol results in an improved oxygen supply-and-demand balance.

Conclusions

The question then remains: Does butorphanol administration ultimately provide any benefit? It is my opinion that butorphanol should still be routinely administered. Although butorphanol did not increase DO_2 or decrease lactate concentration, it did increase $P\bar{v}O_2$ and thus, $S\bar{v}O_2$ and $C\bar{v}O_2$, to near-normal values, indicating an improved balance between DO_2 and VO_2, primarily due to the decrease in VO_2. This balance is likely even more precarious in rhinoceros darted in the field from a helicopter, which run for a variable distance before immobilisation. The additional VO_2 imposed by the muscles of locomotion almost certainly worsens the oxygen supply-and-demand imbalance, which explains the severe hyperlactatemia typically observed in the field. Advanced age, pregnancy, illness, or injury inflicted by poachers would likely do the same. Mixed venous oxygen partial pressure cannot be routinely measured in the field, therefore butorphanol administration likely provides a clinically important margin of

safety that could mean the difference between recovery from immobilisation and morbidity or mortality. However, the effects of oxygen supplementation in addition to butorphanol on DO_2 should be studied further, and until then, I also recommend oxygen supplementation.

CHAPTER 5 DISCUSSION, FUTURE DIRECTIONS, AND CONCLUSIONS

Discussion

Hypoxaemia is arguably the most significant physiological derangement observed in the white rhinoceros during etorphine immobilisation, and research to this date has focused on improving arterial oxygen partial pressure (PaO_2). For the etorphine study (Chapter 3), I hypothesised that 1) mechanisms other than opioid-induced hypoventilation contribute to the hypoxaemia observed in etorphine-immobilised white rhinoceros, and 2) etorphine immobilisation induces sympathetic upregulation that decreases over the period of immobilisation. For the butorphanol study (Chapter 4), I hypothesised that butorphanol would increase oxygen delivery (DO_2) to tissues. The data presented in this thesis show that hypoxaemia during etorphine immobilisation is due not only to hypoventilation but to venous admixture, and is exacerbated by a mixed venous hypoxaemia caused by increased tissue oxygen consumption (VO_2). The data also demonstrate that etorphine induces sympathetic nervous system (SNS) upregulation, resulting in increased plasma noradrenaline concentrations. However, surprisingly, although butorphanol increases PaO_2, the data show that butorphanol does not uniformly increase DO_2; the decrease in cardiac output (Qt) following butorphanol administration negated the increase in arterial oxygen content (CaO_2) in some rhinoceros, which ultimately decreased their DO_2. Nevertheless, I still recommend the administration of butorphanol immediately after contact with an etorphine-immobilised rhinoceros is made, as the butorphanol did increase DO_2 in some rhinoceros and, importantly, decreased VO_2 in all of them, resulting in an improved oxygen supply-and-demand balance. It is likely that combining butorphanol administration with oxygen supplementation, which resolved hypoxaemia in boma-habituated white rhinoceros in the study by Haw et al. (2014), would increase DO_2 to some extent in all rhinoceros; a hypothesis which is left to a future study.[6] My findings should be

extrapolated to field-immobilised, free-ranging white rhinoceros with caution. The physiological pressure of exertion and psychological stress triggered by the helicopter in the field (in addition to the effects of etorphine) likely further upregulate the SNS. In this situation, butorphanol, even with oxygen insufflation, may be insufficient to increase DO_2 and decrease VO_2 adequately. Indeed, Haw et al. (2015) found that butorphanol plus oxygen insufflation *improved* PaO_2 in field-immobilised white rhinoceros but did not *resolve* the hypoxaemia.[5] The challenges experienced when inserting pulmonary arterial catheters (PAC) in bomas makes it unlikely that this study could be repeated in a field setting, however.

It is possible that etorphine-induced hypoventilation and venous admixture secondary to recumbency produced the critical hypoxaemia (and hypercapnia) observed in these rhinoceros. The hypoxaemia (and hypercapnia) could have then triggered noradrenaline release, resulting in the observed increase in plasma concentrations above those recorded in other resting mammals, as well as tremors and other cardiovascular and metabolic evidence of sympathetic upregulation (tachycardia and increased Qt, pulmonary pressures, systemic arterial pressure (SAP), and VO_2. However, it is also possible that noradrenaline release (sympathetic upregulation) was the seminal event, triggered directly by etorphine's action in some central noradrenergic (CNA) site in the brain; the noradrenaline release could have caused the hypoxaemia by increasing blood flow through the pulmonary vascular bed. The increased right-sided Qt could cause gas-diffusion-impairment-induced hypoxaemia by decreasing erythrocyte transit time through the pulmonary capillaries or by producing interstitial oedema, just as it does in human and equine athletes, due to an increase in pulmonary capillary hydrostatic pressure (Pc).[168; 178; 179; 181; 253] Thus, we are left asking ourselves "Which came first? The chicken or the egg?" (i.e., "The hypoxaemia or the noradrenaline release?") In reality, both scenarios likely occur to some extent

in the etorphine-immobilised white rhinoceros. A crossover study consisting of a control treatment and one in which rhinoceros are intubated, ventilated (to produce normocapnia), and administered supplemental oxygen (to produce normoxaemia) could answer this question. If this were the case, I would expect that post-intubation, rhinoceros would have significantly lower plasma noradrenaline concentrations, as well as lower heart rate (f_H), Qt, pulmonary pressures, SAP, and VO_2, in the latter treatment vs. the control treatment. Obviously, my study was not designed to test this hypothesis. My study was also not designed to test whether etorphine is capable of directly triggering noradrenaline release in the rhinoceros brain, and it is unlikely that such a study would ever be possible.

Nevertheless, based on research in other species as well as my data (and that of other investigators) from white rhinoceros, I propose that hypoxaemia did not cause the noradrenaline release (or was at least not solely or primarily responsible for it) and that direct noradrenaline release by etorphine is responsible for most of the physiological derangements observed. Hypoventilation (and resultant hypercapnia) and hypoxaemia can certainly increase noradrenaline concentration and sympathetic outflow in other species.[226; 227] However, this increase is relatively minor when compared to the plasma noradrenaline concentrations observed in these rhinoceros. Even humans with diagnosed phaeochromocytoma do not achieve such high plasma noradrenaline concentrations.[225] These differences suggest that another mechanism is predominantly responsible for the noradrenaline release. A large body of scientific evidence shows that opioids are capable of exerting profound effects on the autonomic nervous system (ANS), with the specific effects dependent on the opioid and the species studied, among many other factors.[9] Therefore, my hypothesis that etorphine has a unique effect on the white rhinoceros and its relative, the domestic horse or pony, seems plausible. Why the black

rhinoceros, which is more closely related to the white rhinoceros than the domestic horse, is not affected as profoundly by etorphine remains to be determined.[254]

Catecholamines are capable of inducing tremors; adrenaline exerts this effect by binding to β_2 receptors.[245; 255; 256] Noradrenaline, although not as potent an agonist at the β_2 adrenoceptor as adrenaline, still has some agonist effect.[256] Although physiological concentrations of noradrenaline may not agonise the β_2 adrenoceptor sufficiently to induce tremors, the high concentrations detected in these rhinoceros may be sufficient.[256] Etorphine may also cause tremors by inhibition of dopamine release in the striatum, pons, cerebellum, and lumbosacral spinal cord.[228] Regardless of mechanism, these tremors may stimulate types III and IV afferent neurons in skeletal muscles, which ultimately communicate with the rostral ventromedial medulla (RVLM).[126; 127; 130] The RVLM may then trigger the 'exercise pressor reflex', which would further increase sympathetic outflow, worsening tremors and creating a vicious cycle.[81] Tremors would also increase VO_2 dramatically, although the increased noradrenaline concentrations could directly increase general tissue metabolism (VO_2).[88] The increase in VO_2 could reduce PʊO2 and CʊO2, which would in turn exacerbate arterial hypoxaemia from other causes.

Future Directions

It may prove difficult to develop a means of *preventing* CNA stimulation and systemic noradrenaline release by etorphine in white rhinoceros. The precise mechanism by which this occurs remains elusive, and conducting studies in white rhinoceros like the ones discussed in Chapter 1 in rats, which involved microinjection of opioids into discrete brain sites, would be impossible.[8; 9] However, different drugs are capable of affecting the release of different catecholamines from the central nervous system (CNS); for instance, it has been shown that

dopamine agonists decrease adrenaline release in rat hypothalamus.[257] Differential effects of drugs on catecholamine release may account for why de Lange et al., who used azaperone (which has antidopaminergic effects) in combination with etorphine measured high plasma concentrations of adrenaline but not noradrenaline, whereas I measured high plasma concentrations of noradrenaline but not adrenaline in this study.[7] The α_2 adrenoceptor agonists, like medetomidine, which is sometimes combined with etorphine for white rhinoceros immobilisation, are central sympatholytics that decrease noradrenaline release from the locus coeruleus (LC).[258] This effect could prove to be useful against etorphine-induced central sympathetic outflow. However, the initial peripheral vasoconstriction that occurs in other mammals after α_2 adrenoceptor agonist administration is a concern, as it might further increase pulmonary and systemic pressures.[259] The initial reflexive decrease in f_H, however, might have beneficial effects.

A preventative or therapeutic intervention that antagonises adrenoceptors might prove useful. Propranolol is predominantly a β_1 adrenoceptor antagonist with considerable β_2 antagonist effects.[260] It mitigates adrenaline-induced tremors in people.[255] Propranolol should also decrease f_H and Qt. A decrease in Qt might decrease Pc and facilitate resolution of pulmonary oedema; it might also increase erythrocyte transit time through the pulmonary artery. It is available in oral and parenteral forms and has been studied in exercising horses, in which it decreases f_H and pulmonary artery blood flow velocity.[261] An α adrenoceptor antagonist, such as phenoxybenzamine, prazosin, or phentolamine, might be combined with a β adrenoceptor antagonist. Phenoxybenzamine is administered orally to humans and dogs with phaeochromocytoma prior to surgery to decrease the risk of serious hypertension intraoperatively.[262; 263] Only an oral formulation is available, which would have to be

administered before darting (hence only practical in a boma). However, azaperone is already used for α adrenoceptor antagonism and can cause hypotension that might compromise perfusion of the dependent limbs and lead to myopathy or neuropathy.[2] Therefore, additional α adrenoceptor antagonism may be dangerous. Furthermore, rather than simply decreasing SAP, azaperone improves the quality of immobilisation through its effects on the CNS and thus may be preferable to a pure vasodilator.

Diuretics such as furosemide are part of the treatment for acute pulmonary oedema in people and begin to act rapidly after intravenous (IV) administration.[181] Furosemide has been shown to decrease the severity of exercise-induced pulmonary haemorrhage in racehorses.[264] If oedema does contribute to the hypoxaemia observed in etorphine-immobilised rhinoceros, an IV bolus of furosemide, for instance, along with butorphanol as soon as contact with a rhinoceros is made, might help clear the pulmonary interstitium of fluid.

Another approach might involve the administration of a muscle relaxant such as midazolam or guaifenesin. The combination of etorphine plus midazolam has been studied in white rhinoceros and appeared to eliminate tremors; however, this study lacked a control group and was conducted in game-ranched white rhinoceros, which may not have tremored much regardless of the adjunctive drug administered with the etorphine.[11] Alleviation of tremors might break the 'vicious cycle' proposed above whereby muscle contraction stimulates the RVLM, which then further stimulates the SNS; it would also decrease VO_2.

Conclusions

In conclusion, this research demonstrates that etorphine produces hypoxaemia not only by causing hypoventilation, but by causing venous admixture. The precise mechanism responsible for the venous admixture remains unclear. However, the strong resemblance of the

cardiopulmonary effects observed in the rhinoceros in this study to those documented in maximally exercising humans and horses leads me to conclude that the same pathophysiological processes likely underlie the hypoxaemia observed in both species: gas diffusion limitation from decreased erythrocyte transit time through the pulmonary capillaries, and interstitial pulmonary oedema. A mixed venous hypoxaemia caused by increased VO_2, likely due in large part to tremors, exacerbates the arterial hypoxaemia. Furthermore, this research demonstrates that etorphine induces sympathetic outflow, resulting in increased plasma noradrenaline concentrations. The nordarenaline release is likely due in part to hypoxaemia and hypercapnia, but direct stimulation of the CNA system probably plays a substantial role. Finally, the research shows that although butorphanol alone may not uniformly increase DO_2 in all rhinoceros, it does improve mixed venous oxygen content ($C\bar{v}O_2$), indicating an improved oxygen supply-and-demand balance, and for this reason should still be administered, ideally with concurrent oxygen supplementation.

This research contributes to our understanding of of the complex constellation of cardiopulmonary and metabolic effects first observed in the etorphine-immobilised white rhinoceros over a half century ago and opens avenues for further research into preventative or therapeutic measures to mitigate these effects. This threatened species is unlikely to survive the current poaching crisis without intensive management by humans, and management is virtually impossible without chemical immobilisation. Etorphine will likely remain one of the only drugs available to wildlife veterinarians for rhinoceros immobilisation for the forseeable future, forcing us to find ways to deal with its adverse effects. Determining the culpable pathophysiological mechanisms is key to mitigating them and improving the safety of chemical immobilisation.

Thus, my findings will surely make a substantial contribution to the field of wildlife medicine, and to the conservation of the species.

CHAPTER 6 MATERIALS AND METHODS SUPPLEMENT

Pulmonary Arterial Catheters: Design and Bench Testing

Design

I collaborated with Gaeltec Devices (Isle of Skye, Dunvegan, Scotland) to design four custom pulmonary arterial catheters (PAC). The PAC were then manufactured by Gaeltec Devices and bench-tested by both the manufacturer and investigators (Figure 6-2, Figure 6-3, Figure 6-4, Table 6-1). These catheters retained the general design of a typical PAC and was similar to Gaeltec Devices 'off-the-shelf' Swan-Ganz catheters, http://www.gaeltec.com/veterinary/Swan-Ganz/#QL-0128C); however, I specified adjustments that would enable my colleagues and I to achieve the goals of our study. For instance, the length of the catheter was extended from 180 cm to 200 cm after the study described in Chapter 2 because my colleagues and I were not able to wedge the balloon in that study. The size of the balloon was also increased from 12 mm to 20-30 mm (inflated) to account for the larger diameter of the branches of the rhinoceros' pulmonary artery. To ensure Gaeltec Devices' PAC communicated with my cardiac output monitor, connectors from another brand of PAC known to be compatible with the monitor were used in the construction of the PAC for my study. The catheter is pictured in Figure 6-1, and the specifications are listed in Box 6-1.

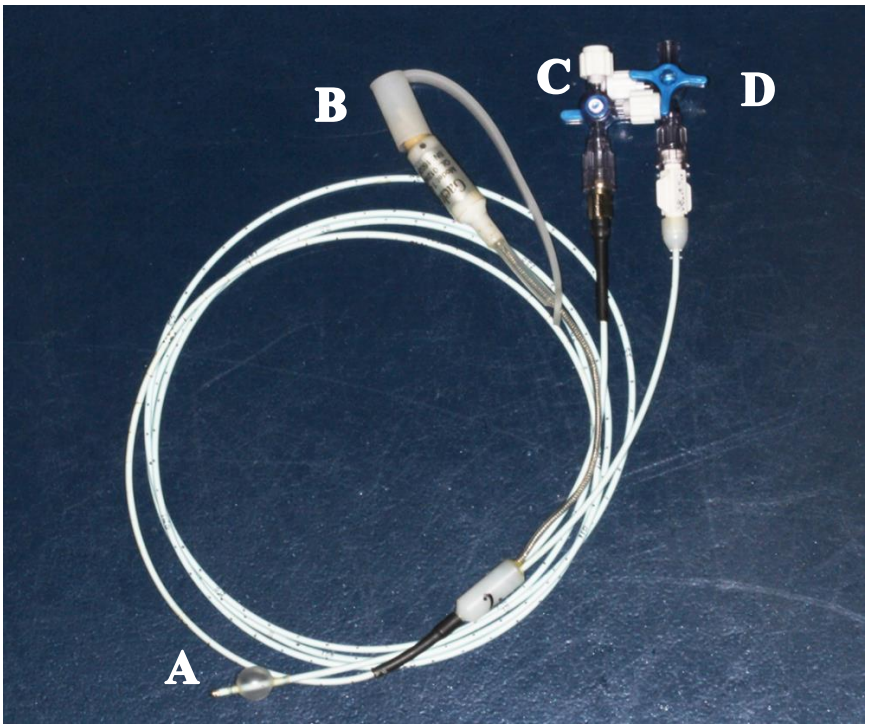


Figure 6-1 The custom pulmonary arterial catheter (PAC). A) Inflated latex balloon, B) connection to cardiac output monitor, (C) connection to lumen for measuring pressure and sampling mixed venous blood, and D) connection to lumen for balloon inflation.

Box 6-1 Custom pulmonary artery catheter (PAC) specifications.

• 200 cm long • 7 Fr (2.33 mm) • Steel braid-reinforced polyurethane • Markings o Lines every 10 cm • Dots every 1 cm	• Thermistor o Exposed to environment at side of catheter just proximal to balloon o Wired to connector (below) o Time constant ~50 milliseconds • Balloon o Made of latex o 15 mm long/0.25 mm wall thickness

• Lumen 1 (0.75 mm internal diameter) with port at distal tip of catheter o Connected to extension line, three-way stopcock, and transducer via luer lock connection at proximal end of catheter o System filled with 0.9% saline o Used for mixed venous blood sampling, and PAP and PAOP measurement • Lumen 2 (0.75 mm internal diameter) o For inflating balloon o Port located 3 cm from tip o Luer lock connection at proximal end of PAC for inflation syringe	o Centered over inflation port o Inflated to ~2-3 cm diameter by injecting pre-determined volume of air[a] o Secured to PAC at each end with a single interrupted suture[b] and replaced as needed if leaking noted • Connector for attachment to cardiac output cable and monitor[c] o Removed from ARROW Balloon Thermodilution catheters[d] and attached to the PAC to ensure compatibility with cardiac output monitor[c]

PAP, pulmonary artery pressure; PAOP, pulmonary arterial occlusion pressure

[a] Inflation volume varied with balloon but was ~3 mL (confirmed prior to each immobilisation by injecting air with a syringe into the pilot balloon until the moment the balloon suddenly inflated). The inflation volume was recorded prior to each immobilisation.

[b] 5-0 monofilament polypropylene suture, Prolene, Ethicon US, LLC, Somerville, New Jersey, USA

[c] PM-9000 Vet Veterinary Portable Multi-Parameter Patient Monitor, ShenZhen Mindray Bio-Medical Electronics Co., Ltd., Nanshan, ShenZhen, China

[d] Teleflex, Morrisville, North Carolina, USA

Bench Testing

Thermistor

Before data collection a thermistor of the type to be used in the custom PAC was bench-tested to determine the accuracy of the temperature (T, °C) values it produced. The test thermistor was mounted in a short length of catheter (~500 mm long) and wired to a connector from an ARROW Balloon Thermodilution catheter (Teleflex, Morrisville, North Carolina, USA). The test thermistor was then connected to the cardiac output cable, which was connected to the cardiac output monitor (PM-9000 Vet Veterinary Portable Multi-Parameter Patient Monitor, ShenZhen Mindray Bio-Medical Electronics Co., Ltd., Nanshan, ShenZhen, China).

A circulating warm water bath was made using a heating element with a rotary knob (Polystat Immersion Circulator, MDL 1266-30, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA) that controlled the T of the water bath. The water bath T was initially set at 20°C using the rotary knob. The T probe on the cardiac output monitor (used for measuring rectal or esophageal T and distinct from the test thermistor) and the T probe on a second physiological monitor (Cardell Touch Veterinary Monitor, Model 8013-003, Midmark Corporation, Versailles, Ohio, USA) were used as ‘standards’. These were bundled with the test thermistor and submerged to an equal depth in the water bath. The three T values registered on the two monitors were allowed to stabilise and were then recorded. The heating element was used to increase the T of the water bath by 5°C, and after stabilisation at each bath T, values were again recorded.

Linear regression of the two ‘standards’ using commercial software (GraphPad Prism 7 for Windows, version 7.03, 2017, GraphPad Software, Inc., La Jolla, California, USA) produced a slope (m) = 1.008 ± 0.008008, R^2 = 0.997, and Y intercept (b) = 0.1383 ± 0.282 (Figure 6-2).

Because there was a slight difference (i.e., m > 1.0) between the two monitors, and because I was unsure which monitor was more accurate, I regressed the T measured by the test thermistor against the mean of the two values T measured by the two monitors' probes at each water bath T. The linear regression produced m = 1.093 ± 0.05851, R^2 = 0.9915, and b = -3.903 ± 2.08 (Figure 6-3).

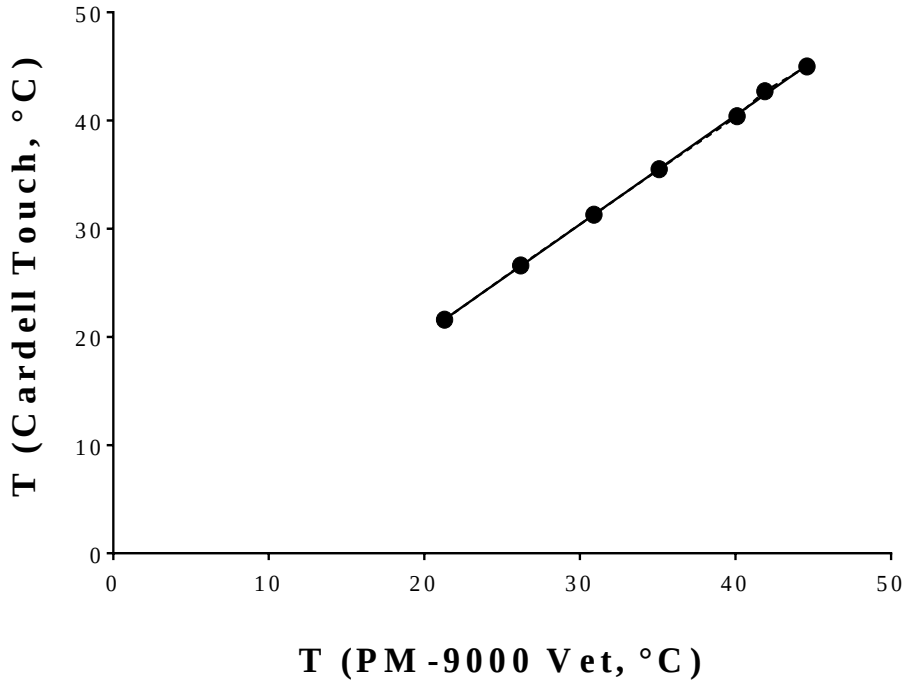


Figure 6-2 Scatter plot of temperature (T, °C) measured by the T probe on the Cardell Touch vs. the PM-9000 Vet. The solid line represents the linear regression line.

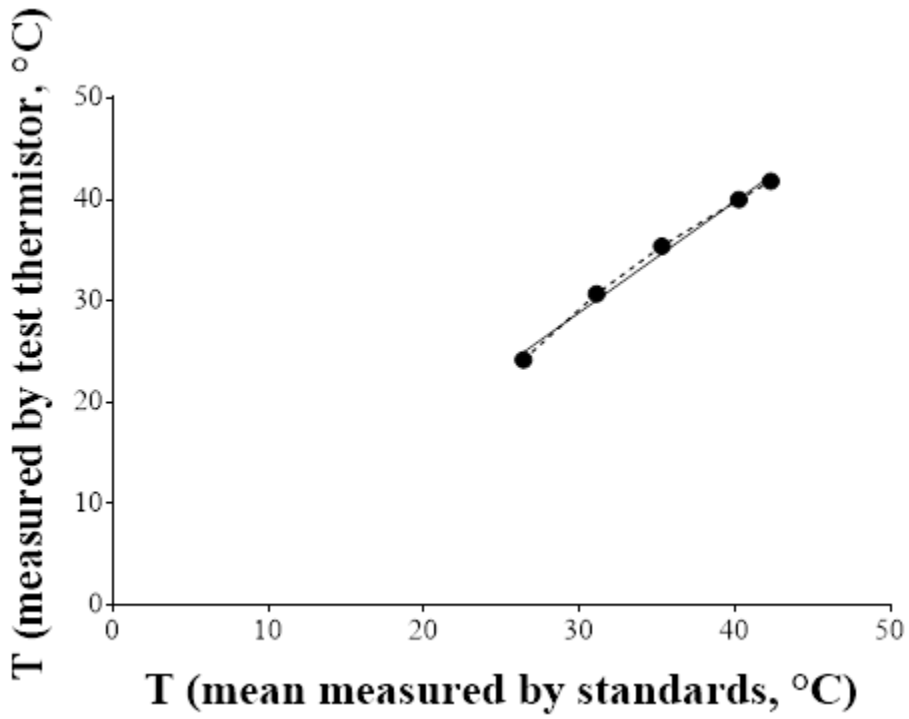


Figure 6-3 Scatter plot of temperature (T, °C) measured by the test thermistor vs. the mean T (°C) measured by the two standards, the Cardell Touch and the PM-9000 Vet. The solid line represents the linear regression line.

After data collection, the accuracy of the thermistors incorported into the 4 PAC used for data collection was evaluated. Separately, each thermistor was suspended in a circulating water bath at the same depth as a National Institute of Standards and Technology (NIST) certified thermocouple (#EVL588304, Fluke 80TK Thermocouple Module, Fluke Corporation, Everett, Washington, USA). The water bath was heated over the range recorded for pulmonary artery temperature in the rhinoceros (36.0-46.0°C), and the contemporary temperatures measured by the certified thermocouple and the thermistor in the PAC were recorded; 36 was subtracted from each value. The resulting values for each PAC were then graphed (Figure 6-4). The regression equations are listed in the caption.

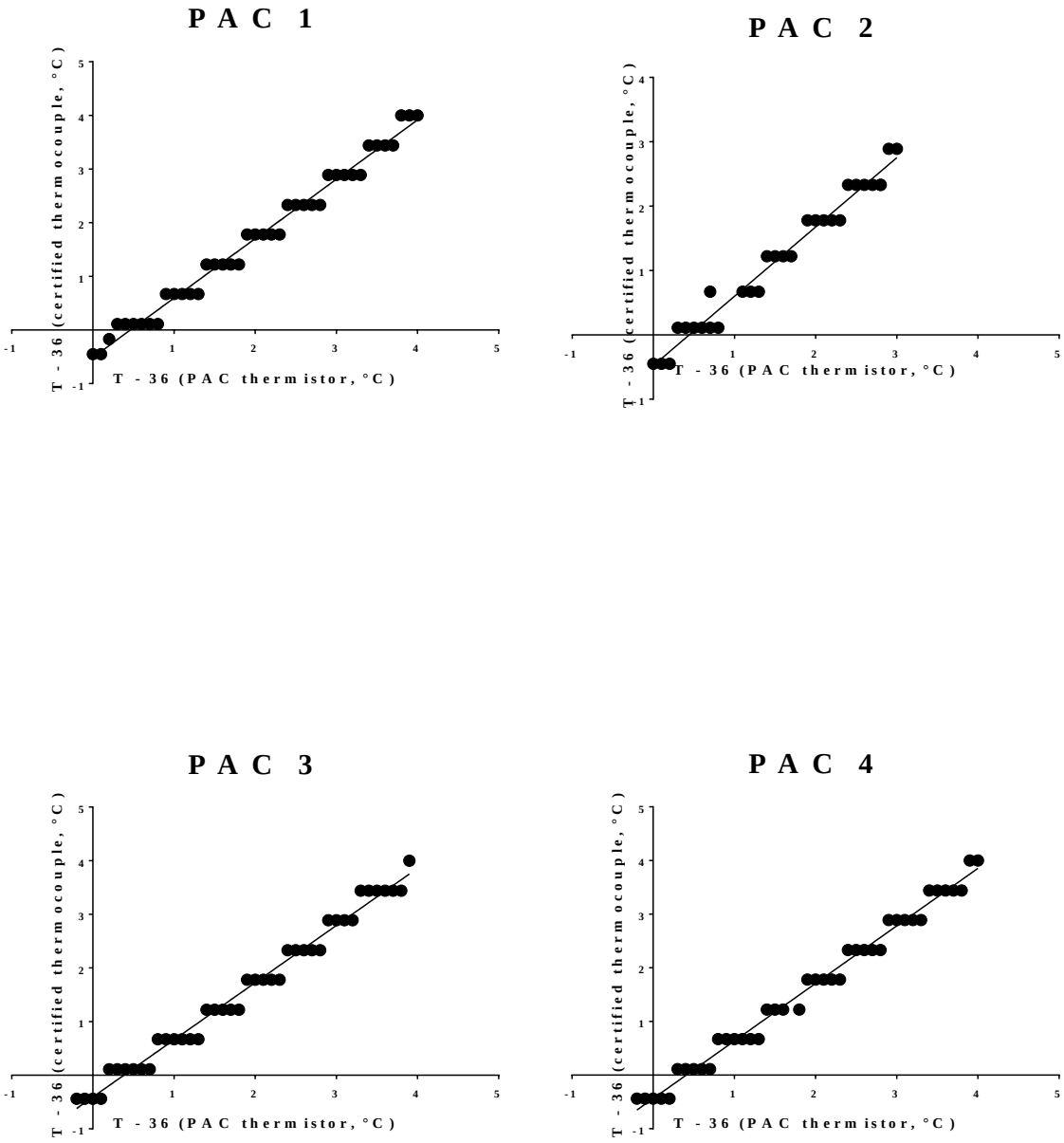


Figure 6-4 Temperature (T, °C) – 36 recorded by each of the 4 pulmonary artery catheter (PAC) thermistors used for data collection (X axis) and contemporary values recorded by a certified thermocouple (Y axis), both suspended in a water bath. The equations for each line are Y = 1.107 * X - 0.5119, R^2 = 0.9849 (PAC 1), Y = 1.077 * X - 0.4772, R^2 = 0.9849 (PAC 2), Y = 1.066 * X - 0.4069, R^2 = 0.983 (PAC 3), and Y = 1.073 * X - 0.4376, R^2 = 0.9858 (PAC 4).

Pressure-Measuring Assembly (Catheter, Transducer, Tubing, and Stopcocks)

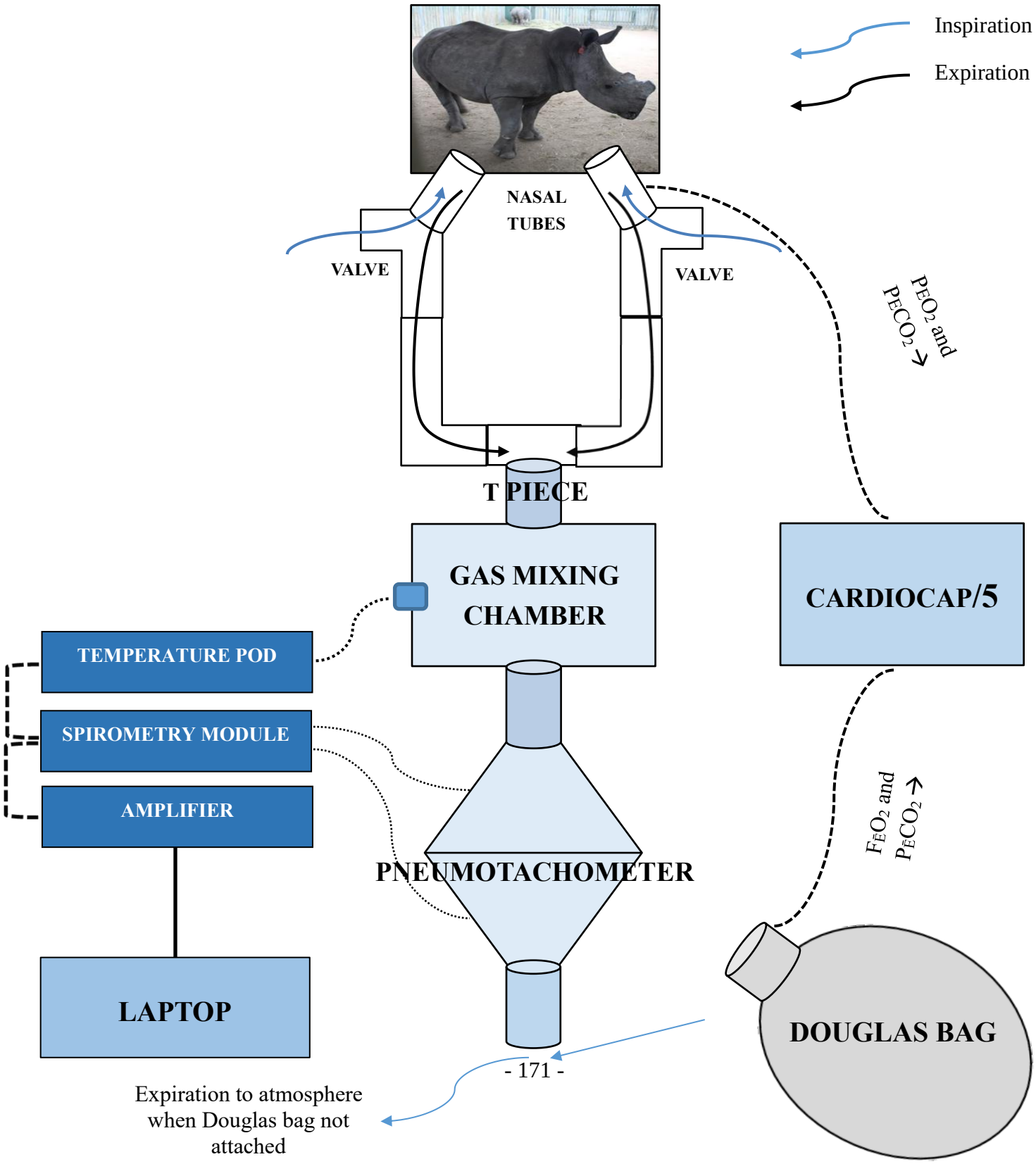
We tested the fidelity of each of the four PAC assemblies by measuring their damping characteristics. In each case, I inflated a small balloon over the tip of the catheter and punctured it with a hot needle while recording the pressure waveform at a sampling rate of 1,000 Hz (LabChart 7, version 7.3.8, ADInstruments Pty Ltd, New South Wales, Australia). This test was repeated 3-4 times for each catheter assembly. The pressure approached atmospheric pressure (P_B) rapidly after puncture, but oscillations were not observed after balloon puncture in any catheter assembly, precluding measurement of damping coefficients (Table 6-1) and showing that the catheters were all overdamped. The tubing from which the catheters are made is usually used in human medicine in catheters that are ~110 cm long; under those circumstances, they approach critical damping and can measure a pressure waveform oscillating at physiological rates with high fidelity. My catheter assemblies were probably overdamped because the PAC themselves were so much longer than those used medically. Overdamping is likely to cause underestimation of systolic pressure and overestimation of diastolic pressure but is unlikely to bias mean vascular pressure. This thesis reports mean vascular pressure exclusively. The decay in the pressure waveform after balloon puncture allowed me to measure time constants (τ, tau) for each catheter using the LabChart 7 software. These τ were ~0.1 second for each catheter (Table 6-1) and suggested that they had response times that were at least sensitive enough for me to be able to identify the characteristic pressure waveforms when the tip of the catheter was in the right ventricle, pulmonary artery, or occluding a branch of the pulmonary artery.

Table 6-1 Damping characteristics of the four pulmonary arterial catheters (PAC).

PAC #	Test #	Oscillations	Pressure before bursting (mm Hg)	τ (sec)	Median τ (sec)
1	1	no	25.6	0.056	
1	2	no	31.2	0.074	0.072
1	3	no	31.1	0.072	
2	1	no	45.3	0.093	
2	2	no	41.4	0.072	0.093
2	3	no	22.7	0.100	
3	1	no	27.7	0.112	
3	2	no	26.4	0.103	0.103
3	3	no	26.0	0.093	
4	1	no	30.6	0.096	
4	2	no	28.3	0.126	0.119
4	3	no	34.0	0.119	

τ, tau (time constant); sec, seconds

Figure 6-5 Schematic of the breathing system connected to the shortened equine endotracheal tubes inserted into the white rhinoceros' nares.



Computer-Based Data Acquisition and Extraction

The basic principles behind computer-based data acquisition are discussed by the *Welcome Centre* and under the *Help* tab in LabChart 7. Briefly, computer-based data acquisition begins with a transducer converting a signal (e.g., blood pressure) into an analogue voltage with an amplitude that varies over time. This voltage is received by the recording PowerLab hardware (ADInstruments Pty Ltd) where it is modified by amplification and filtering; this process is called ‘signal conditioning'. The signal is then sampled at regular intervals and converted from analogue to digital form. When the PowerLab hardware digitises the signal, it records the voltage of the analogue signal multiple times each second; this ‘sampling rate’ is adjustable by the user, but these data were collected at a default sampling rate of 1,000 data points second^{-1}. The digitised signal is transmitted to a computer using a USB connection. The computer software plots the sampled and digitised data points and reconstructs the original waveform by interpolating between the points.

One LabChart 7 file was created for each of the 12 immobilisations. The *Chart View* window was opened, and the channels which displayed waveforms for systemic arterial pressure (SAP), pulmonary pressure, and expiratory flow were displayed. These waveforms were used to determine values for mean SAP (mSAP), mean PAP (mPAP), PAOP, minute ventilation ($\dot{V}_E$), and respiratory frequency (f_R). Sampling points were identified during data collection by typing a *Comment* (*Sample 30*, *Sample 40*, or *Sample 50*) at time (t) = 30, 40, or 50 minutes (t = 30, 40, or 50), respectively. Rules were established *a priori* for extracting values from the LabChart 7 files for subsequent analysis (Box 6-2); the rules were principally designed to ensure consistency and accuracy, and to minimise the opportunity for operator bias. Relevant periods of waveform for analysis were determined by the operator by applying these rules. Each period of waveform for

analysis was 'highlighted' and the variables of interest for that period (e.g., mean vascular pressures or V_E) calculated using LabChart 7 software routines. Those values were transferred to a digital spreadsheet (Microsoft Excel) for subsequent statistical analysis.

Box 6-2 Rules for LabChart 7 data extraction.

- Identify the sampling points of interest (t = 30, 40, or 50) in the data file.
- Measuring mPAP and mSAP at each sampling point
  - o Using the expiratory flow waveform, highlight two complete respiratory cycles that occur within the minute immediately before the data collection time. This automatically highlights the contemporary pulmonary and SAP waveforms. For this purpose, a respiratory cycle is usually defined as 'peak expiratory flow to peak expiratory flow', although occasionally it can be defined as 'start of expiratory flow to start of expiratory flow'.
  - o Ensure high-quality PAP and SAP waveforms coincide with the highlighted region, and, particularly, check that the PAC is not wedging. If either signal is questionable then an alternative pair of respirations within the minute are chosen and highlighted.
  - o Press the LabChart 7 command Ctrl D, which calculates mPAP and mSAP for the highlighted period and saves them in the *Data Pad* (i.e., LabChart 7 spreadsheet).
- Measuring PAOP at each sampling point
  - o Highlight one or two complete repiratory cycles after the PAP has decreased subsequent to balloon inflation.
  - o Press the LabChart 7 command Ctrl D which calculates mPAOP for the highlighted period and saves it in the *Data Pad* (i.e., LabChart spreadsheet).
- Measuring V_E and f_R at each sampling point

- Highlight the expiratory flow waveform beginning at the start of the first expiration after the sampling point. Continue highlighting for the 60 + X seconds needed to reach the start of the first expiration after a minute has passed. This highlighted period also corresponds closely to the minute over which expired gas was collected in the Douglas bag.
  - This approach allows f_R to be calculated by dividing the number of breaths in the highlighted period by the duration of the highlighted period (60 + X seconds); this value is in units of breaths second^{-1} and can be converted to breaths minute^{-1} by multiplying by 60.
- In LabChart 7, click the *Metabolic* tab, then the *General* tab. Set averaging time to the minimum (10 seconds) and click *OK*. A spreadsheet appears that gives a value for V_E every 10 seconds during the highlighted period (i.e., 6-7 values). The spreadsheet is copied into a Microsoft Excel spreadsheet. The mean of the 6-7 V_E values is taken as V_E at each sampling point. Note that V_E is given as BTPS.

PAP, pulmonary arterial pressure; SAP, systemic arterial pressure; t, time; PAOP, pulmonary arterial occlusion pressure; mPAOP, mean pulmonary arterial occlusion pressure; V_E, minute ventilation; f_R, respiratory rate, BTPS, body temperature and pressure saturated with water vapour.

Calculation of Blood Oxygen Content

Oxygen content is calculated using the following equations (Box 6-3).[243]

Box 6-3 The arterial and mixed venous oxygen content (CaO_2 and $C\bar{v}O_2$) equations.

$$CaO_2 \text{ (mL dL}^{-1}) = ([Hb] * 1.39 * SaO_2) + (PaO_2 * 0.003)$$

$$C\bar{v}O_2 \text{ (mL dL}^{-1}) = ([Hb] * 1.39 * S\bar{v}O_2) + (P\bar{v}O_2 * 0.003)$$

[Hb], concentration of effective haemoglobin in blood (g dL^{-1}), which = concentration total haemoglobin (*ct*Hb) – concentration of non-oxygen-carrying dyshaemoglobins (*cdys*Hb, not measured in this study and assumed to be 0)

1.39 = oxygen binding capacity of haemoglobin (mL O_2 g^{-1} haemoglobin), or "Hüfner's factor" (β)

SaO_2 or $S\bar{v}O_2$, saturation of haemoglobin, in arterial or mixed venous blood, with oxygen (%)

PaO_2 or $P\bar{v}O_2$, arterial or mixed venous oxygen partial pressure (mm Hg)

*, multiplied by

0.003 = solubility constant for oxygen in serum (mL O_2 mm Hg O_2^{-1} dL $blood^{-1}$) at 37°C

Haemoglobin Concentration

Accurate determination of haemoglobin (Hb) concentration ([Hb]) is necessary for calculating blood oxygen content. The international standard for measurement of [Hb] is the cyanmethaemoglobin method, although other methods and analysers are available.[265; 266] However, none were available for measurement of rhinoceros [Hb]. The epoc Blood Analysis System (Siemens Medical Solutions Inc., Malvern, Pennsylvania, USA) used to measure blood gases calculates [Hb] from haematocrit (Hct) using the equation [Hb] (g dL^{-1}) = Hct (decimal fraction) x 34, which assumes a normal mean corpuscular [Hb] (MCHC) of 34 g dL^{-1}; Hct is measured by this system using AC conductometry.[267] However, this technique has not, to my knowledge, been validated in the white rhinoceros and conductivity might vary by species depending on the conformation of erythrocytes.

In each rhinoceros in this study, packed cell volume (PCV) of arterial and mixed venous blood was determined at each of the three sampling points by centrifuging a portion of the arterial and mixed venous blood samples at 15,000 g for 10 minutes. The PCV values were often markedly different from the haematocit (Hct) values from the epoc Blood Analysis System, raising concern that the Hct values, and thus, the values for [Hb] calculated from them, were inaccurate.

For this reason, I devised an equation for calculation of [Hb] from PCV in the white rhinoceros. From 2013-2017, 214 sub-adult, male white rhinoceros were captured in Kruger National Park. On a sample of blood from each animal, the PCV was determined as described

above, while [Hb] was measured with the cyanmethaemoglobin method using an automated haematology analyser (Vet abc, scil animal care company, Gurnee, Illinois, USA). A regression line (described by the equation y = mx + b, with m = slope and b = Y intercept) for PCV vs. [Hb] was generated using a commercial software package (GraphPad Prism 7, GraphPad Software Inc., San Diego, California, USA) (Figure 6-6); this line was described by the equation y = 0.2528x + 4.013. The value for R^2 was 0.535. This equation was used to calculate [Hb] in both arterial and mixed venous samples at each sampling point in this study.

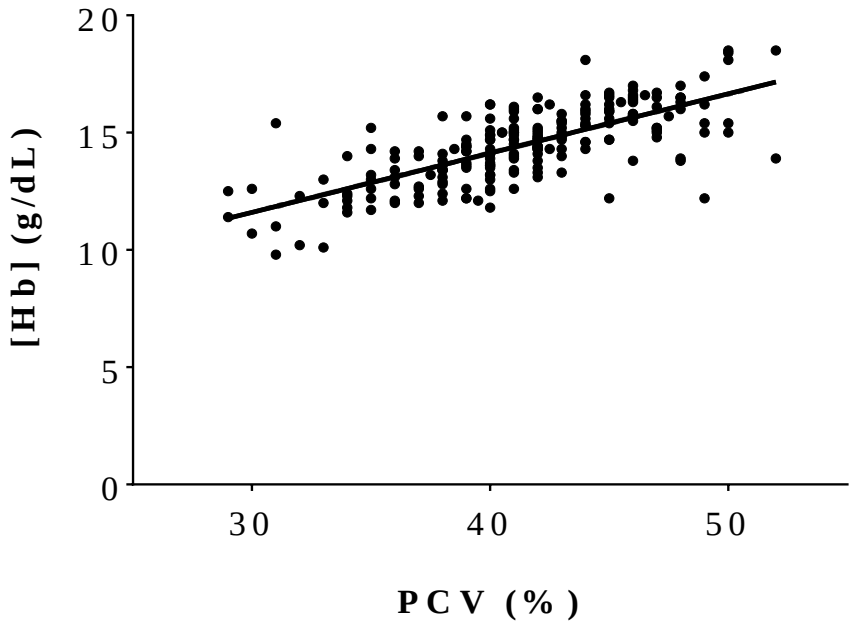


Figure 6-6 Scatter plot of haemoglobin concentration ([Hb]) obtained using the cyanmethaemoglobin assay vs. packed cell volume (PCV) for 214 sub-adult, male white rhinoceros captured in Kruger National Park (KNP) from 2013-2017. The solid line represents the linear regression line described by the equation y = 0.2528x + 4.013.

Oxygen Binding Capacity of Haemoglobin

The oxygen binding capacity of Hb ('Hüfner's factor' or β) is, to my knowledge, unknown for the white rhinoceros. The value for β is the volume of oxygen (in mL) bound to 1 g of haemoglobin and is quoted variably in textbooks as 1.30-1.39 mL O_2 g Hb^{-1} for other species. The value of 1.39 that I use represents the 'ideal' or 'maximal' oxygen-carrying capacity of Hb (the volume carried if every last Hb molecule bound four molecules of oxygen) calculated using stoichiometry.[268] To my knowledge, this value is unknown for the white rhinoceros; however, it is likely based on in vitro experiments using human blood that the actual value is somewhat lower. Values for the rhinoceros' CaO_2 recalculated using 1.30 for β would have differed by less than 1 mL dL^{-1}, however.

Oxyhaemoglobin Saturation

The function (i.e., relationship) between the PO_2 in blood (p) and SO_2 (s) is represented by the familiar S-shaped oxyhaemoglobin dissociation curve (ODC); its parameters are listed in Box 6-4.[238] Siggaard-Andersen et al. published the mathematical model, the 'tanh equation' (pronounced 'tanch', below) representing the ODC in 1984 and expanded upon it in 1988 (Box 6-5).[237; 238] It was derived from Severinghaus' tabulated, experimentally-derived values for oxygen partial pressure (PO_2) and haemoglobin saturation with oxygen (SO_2).[269]

Box 6-4 Parameters of the function between oxygen partial pressure (PO_2) and oxyhaemoglobin saturation (SO_2).

- Abscissa (x coordinate) = ln(p) (the natural logarithm of p, or the PO_2 in kPa)
- Ordinate (y coordinate) = ln(s/1 – s) (the natural logarithm of s/1 – s) where s = SO_2
- Point of symmetry: (p_0, s_0) or (x_0, y_0)

- Slope at the point of symmetry: n_0
- Horizontal or vertical distance between the two asymptotes (the lines which approach the curve but do not reach it): 2 * h

kPa, kilopascals

Box 6-5 Siggaard-Andersen's mathematical model of the oxyhaemoglobin dissociation curve (ODC). Note that the term 'tanh' is not tan * h but rather the 'hyperbolic tangent function', pronounced 'tanch'.

$y = y_0 + x - x_0 + h * \tanh[k * (x - x_0)]$

- y = the y coordinate of the patient's SO_2, or ln(s/1-s)
- y_0 = 1.875
  - The value of 1.875 is the y coordinate at the point of symmetry and is obtained by plugging the value for s_0 (0.867) into ln(s/1 - s).
  - This value remains constant in the model.
- x = ln(p) where p = the patient's measured PO_2
- x_0 = 1.946 + a + b
  - The value of 1.946 is the x coordinate at the point of symmetry and is obtained by plugging the value for p_0 (7 kPa in humans) into ln(p), or ln(7). 'a' and 'b' reflect the effects of the allosteric effectors and T (below).
- h^a = 3.5 + a
- k^a = 0.5343
- a^a = cB * (pH – 7.4) + 0.005 * ln(PCO_2/5.33) + (0.07 – 0.03 * x_{HbF} * [c_{DPG} – 5]) – (0.368 * x_{HbCO}) – (0.174 * x_{Hi}) – (0.28 * x_{HbF})

- The value for ‘a’ depends on the allosteric effectors 1) pH, 2) PCO_2, and 3) concentrations of 2,3-DPG (c_{DPG}), x_{HbF}, x_{HbCO}, and x_{Hi}.[b]
- $cB = \ln(10) * \beta$ where β = the Bohr coefficient[c] (the ratio of the change in $\log_{10}p$ to the change in pH, or $\Delta\log_{10}p/\Delta pH$)

- $b^a = 0.055 * (T - 37)$
  - The value for ‘b’ reflects the effect of T in °C.

kPa, kilopascals; T, temperature; 2,3-DPG (or c_{DPG}), 2,3-diphosphoglycerate; x_{HbF}, fetal haemoglobin; x_{HbCO}, carboxyhaemoglobin; x_{Hi}, methaemoglobin

[a] The values of 3.5, 0.5343, 0.005, and 0.055 in these equations were assumed to remain the same in the white rhinoceros.

[b] The effect of 2,3-DPG on the haemoglobin (Hb) of the white rhinoceros is negligible.[231] I assumed that the concentrations of the various dyshaemoglobins in my study rhinoceros were 0. Thus, the equation for ‘a’ reduces to $a = cB * (pH - 7.4) + 0.005 * \ln(PCO_2/5.33)$ for my study.

[c] The Bohr coefficient of the white rhinoceros was published as -0.62 by Baumann et al. (1984) and is used in this thesis, although more recently Reiners et al. (2019) published a value of -0.74.[231; 232]

By plugging a rhinoceros’ values for pH, PCO_2, PO_2, and temperature (T, in this study, that of the pulmonary arterial blood) into the tanh equation, one can solve for y. The equation $s = e^y/(1 + e^y)$ is then used to calculate SO_2.

However, the white rhinoceros has a lower P_{50} than humans (i.e., the white rhinoceros’ ODC is left-shifted relative to the human ODC). Baumann et al. (1984) published a P_{50} of 2.31 kPa (or 17.4 mm Hg, at 37ºC, pH = 7.2, and [Hb] = 4 g dL^{-1}), while Reiners et al. (2019) published more recently a P_{50} of 2.75 ± 0.07 kPa (or 20.6 mm Hg, at 37ºC and pH = 7.2); Haymerle et al. (2016) used a value of 2.67 kPa (20.0 mm Hg), which is the value used in this thesis and is closer to that determined by Reiners et al (2019).[231-233] The ODC for the white rhinoceros must thus pass through the point (p, s) = (2.67, 0.5), which changes their point of

symmetry (p_0, s_0 or x_0, y_0). The value for y_0 would remain the same, but the value of x_0 would differ (i.e., the value of 1.946 in Box 6-5 would differ). Thus, I had to calculate the x coordinate for their unique point of symmetry, which I designated (x_r, y_r) rather than (x_0, y_0) for clarity, using the values for (p, s) of (2.67, 0.5) (Box 6-6), as previously described.[233]

Box 6-6 Calculation of the point of symmetry of the white rhinoceros oxyhaemoglobin dissociation curve (ODC).

$y = y_r + x - x_r + h * \tanh[k * (x - x_r)]$

Where

- $y = \ln(s/1 - s) = \ln(0.5/1 - 0.5) = \ln(1) = 0$
- $y_r = 1.875$
- x_r = the unknown x coordinate of the white rhinoceros point of symmetry
- $x = \ln(2.67) = 0.973$
- $h = 3.5$
- $k = 0.5343$

Plugging in these values gives

$0 = 1.875 + 0.973 - x_r + 3.5 * \tanh[0.5343 * (0.973 - x_r)]$

Rearranging and solving for x_r gives a value of 1.646 instead of 1.946.

Thus, when calculating y for the white rhinoceros, the equation for x_0 in Box 6-5 becomes x_0 (or x_r) = 1.646 + a + b.

Although some assumptions were made in the SO_2 calculations, until the complete ODC of the white rhinoceros is published, these calculations give the most accurate SO_2 possible at this time. Figure 6-7 demonstrates the substantial discrepancy between SO_2 values calculated as

above and the measured values from the epoc Blood Analysis System (Siemens Medical Solutions, Inc., Malvern, Pennsylvania, USA).

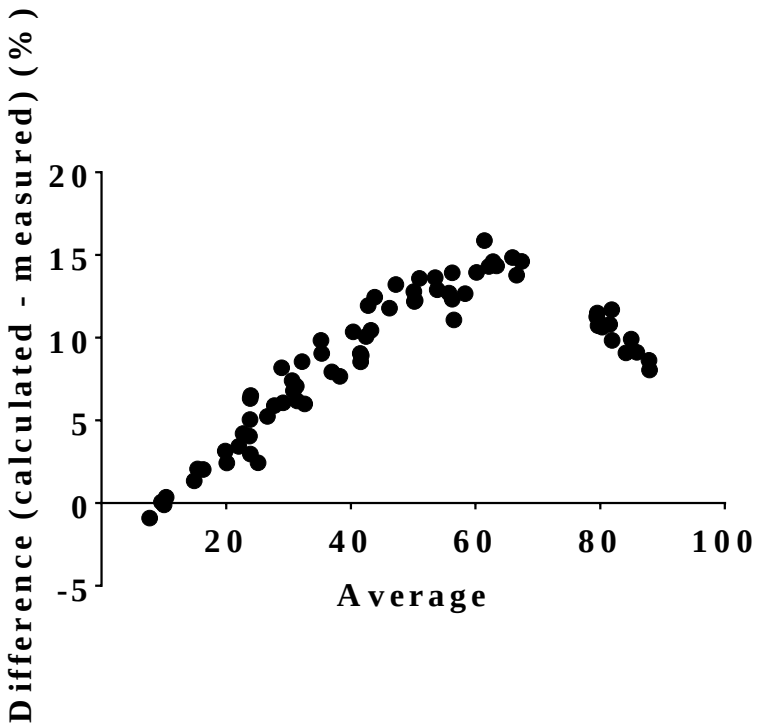


Figure 6-7 Bland-Altman plot of the difference between the oxyhaemoglobin saturation (SO_2) values calculated as described above and the SO_2 values measured by the epoc Blood Analysis System (Siemens Medical Solutions, Inc., Malvern, Pennsylvania, USA), plotted against their average.

The Haldane Transformation

The Haldane transformation is used in the calculation of oxygen consumption (VO_2) (Box 6-7).

Box 6-7 The Haldane transformation.[196]

- $VO_2 = (V_I * F_IO_2) - (V_E * F\bar{E}O_2)$
- We only measured V_E in this study and cannot assume $V_I = V_E$ because RER is not necessarily 1. Therefore, I must first determine V_I.
- The Haldane assumption states that $V_IN_2 = V_EN_2$ because N is inert and is neither produced nor consumed.
    - $V_IN_2 = V_I * F_IN_2$
    - $V_EN_2 = V_E * F_EN_2$
    - Therefore, $V_I * F_IN_2 = V_E * F_EN_2$
    - Now I have a way to solve for V_I; rearranging gives $V_I = (V_E * F_EN_2)/F_IN_2$.
- V_E is measured as BTPS and converted to STPD. $F_EN_2 = 1 - (F\bar{E}O_2 + F\bar{E}CO_2)$, and I measured $F\bar{E}O_2$ and $F\bar{E}CO_2$. F_IN_2 is known to always be 0.79.
- Now, $V_I = (V_E * \{1 - F\bar{E}O_2 + F\bar{E}CO_2\})/0.79$. This value is then plugged into the equation above for calculation of VO_2.

VO_2, oxygen consumption; V_I, inspired volume, * multiplied by; F_IO_2, inspired oxygen fraction, V_E, minute ventilation (or expired volume); F_EO_2, expired oxygen fraction, RER, respiratory exchange ratio; V_IN_2, volume of inspired nitrogen; V_EN_2, volume of expired nitrogen; BTPS, body temperature and pressure, saturated with water vapour; STPD, standard temperature and pressure, dry; F_ECO_2, expired carbon dioxide fraction.

High Performance Liquid Chromatography-Electrochemical Detection (HPLC-ECD)

High performance liquid chromatography-electrochemical detection (HPLC-ECD) was used to detect and quantify plasma noradrenaline, adrenaline, and dopamine concentrations in the white rhinoceros. The assay and laboratory (School of Pharmacy, Faculty of Health Sciences, North West University, Potchefstroom, South Africa) used in this study were identical to those used in another, recently published white rhinoceros study.[7]

Chemical and Reagents

L-Noradrenaline hydrochloride, epinephrine bitartrate salt, dopamine, and 3,4-dihydroxybenzylamine (internal standard) were obtained (Sigma-Aldrich Pty Ltd, Modderfontein, South Africa). Chemicals used for the mobile phase were HPLC-grade deionised water, methanol, and acetonitrile; sodium dihydrogen phosphate buffer; 1-octanesulphonic acid sodium salt; ethylenediaminetetraacetic acid (EDTA) disodium salt; ortho-phosphoric acid (85%); and perchloric acid (70%) (Sigma-Aldrich Pty Ltd).

Materials

The analytical HPLC column used in this study was a Venusil ASB C18 (Bonna-Agela Technologies, Torrance, California, USA), measuring 4.6 * 250 mm, with a particle size of 5 µm, pore size of 300 angstroms (Å), and a surface area of 200 m^2 g^{-1}.

Instrumentation

The chromatographic system consisted of an UltiMate^{TM} 3000 UHPLC system, equipped with an ISO-3100SD isocratic pump and WPS-3000TSL analytical autosampler and coupled to an ECD-3000RS rapid separation ECD with dual channel 6011RS ultra Coulometric Analytical

Cell and Chromeleon™ Chromatography Data System (version 7.2, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Instrumentation Settings

The settings used for the assay are depicted in Box 6-8.

Box 6-8 Settings for high performance liquid chromatography-electrochemical detection (HPLC-ECD) for measurement of plasma catecholamine concentrations in the white rhinoceros.

- HPLC instrument settings
  - Flow rate: 1.0 mL minute^{-1}
  - Injection volume: 25 µL
  - Run time: 25 minutes
  - Column temperature: 23°C (column compartment)
- ECD settings
  - Cell potential settings
  - Test electrode 1 (E1): -250 mV (to eliminate background noise)
  - Test electrode 2 (E2): +650 mV (to analyse the analytes)
  - Detection range: automatic
  - Data collection rate: 20 Hz

Mobile Phase Preparation

A mobile phase consisting of 10 mM sodium dihydrogen phosphate buffer, 4 mM sodium 1-octanesulfonate, 0.17 mM EDTA disodium salt, 6% (v/v) methanol, and 4% (v/v) acetonitrile was prepared. The pH of the mobile phase was set at 4.0 with ortho-phosphoric acid (85%). The mobile phase was filtered through a 0.22 µm nylon filter before use (Agela Technologies).

Sample Preparation

The HPLC method used for analysing noradrenaline, adrenaline, and dopamine concentrations in plasma is a modification of the methods used by De Villiers et al. (1987). Cryotubes, containing the frozen plasma samples were stored in a -80°C freezer until the day of analysis. On the day of analysis, they were placed on ice so as to thaw slowly. Approximately 50 mg of acid-washed alumina (type WA – 4, acid), then 900 µL of plasma, 500 µL Tris buffer and 20 µL of internal standard, with a concentration of 7.5 µg mL^{-1} was added to an empty 1.5 mL Eppendorf Tube (Eppendorf AG, Hamburg, Germany). The Tris buffer created a more alkaline solution, allowing the catecholamines to bind to the alumina. Once all the solutions had been placed into the tube, the mixture was vigorously mixed for 30 minutes. Thereafter, the mixture was centrifuged at room temperature for 10-15 minutes at 20,817 relative centrifugal force (RCF). The supernatant that formed at the top of the tube was then removed with a micropipette and discarded. The alumina that remained in the tube was rinsed twice with 1 mL of double distilled water. This resulted in the formation of supernatant at the surface, which was removed from the tube again. To correct the pH of the alumina, 2,200 µL of 0.1M perchloric acid solution was added. The mixture was then placed on ice for 30 minutes so that the change in pH over time could allow the catecholamines to desorb from the alumina. After the 30 minutes, the mixture was then vortexed and centrifuged for 5 minutes at 20,817 RCF. The entire acidic supernatant sample was cautiously pipetted into a 300 µL insert, which fits into a vial, which in turn was placed into a sample tray of an Agilent 1200 Series autosampler. Twenty-five µL of the sample was programmed to be injected into the column for analysis. The peak area of each catecholamine in the sample was recorded on a spreadsheet and converted to ng mL^{-1} using the

straight line equation (y = mx + b) of the calibration curve of each catecholamine. The calibration curve for each catecholamine ranged from 10-75 ng mL^{-1}.

The Linear Mixed Effect Model

Table 6-2 Parameter estimates given by the linear mixed effect model for each physiological variable.

mPAP (mm Hg)

Term	Estimate	SE	DFDen	t Ratio	Prob > [t]	Lower 95%	Upper 95%
Intercept	54.389	2.790	5.000	19.490	<.0001	47.217	61.561
Phase[Butorphanol]	-2.667	1.416	5.000	-1.880	0.118	-6.305	0.972
Time[30]	5.944	1.403	20.000	4.240	0.000	3.018	8.871
Time[40]	-2.139	1.403	20.000	-1.520	0.143	-5.066	0.788
Time[30] * Phase[Butorphanol]	4.167	1.403	20.000	2.970	0.008	1.240	7.094
Time[40] * Phase[Butorphanol]	-1.417	1.403	20.000	-1.010	0.325	-4.344	1.510

PAOP (mm Hg)

Term	Estimate	SE	DFDen	t Ratio	Prob > [t]	Lower 95%	Upper 95%
Intercept	54.389	2.790	5.000	19.490	<.0001	47.217	61.561
Phase[Butorphanol]	-2.667	1.416	5.000	-1.880	0.118	-6.305	0.972
Time[30]	5.944	1.403	20.000	4.240	0.000	3.018	8.871

Table 6-2, continued

Time[40]	-2.139	1.403	20.000	-1.520	0.143	-5.066	0.788
Time[30] * Phase[Butorphanol]	4.167	1.403	20.000	2.970	0.008	1.240	7.094
Time[40] * Phase[Butorphanol]	-1.417	1.403	20.000	-1.010	0.325	-4.344	1.510

mSAP (mm Hg)

Term	Estimate	SE	DFDen	t Ratio	Prob > [t]	Lower 95%	Upper 95%
Intercept	139.111	8.763	5.000	15.880	<.0001	116.586	161.637
Phase[Butorphanol]	-7.167	5.501	5.000	-1.300	0.249	-21.307	6.973
Time[30]	5.889	4.500	20.000	1.310	0.206	-3.499	15.276
Time[40]	-12.028	4.500	20.000	-2.670	0.015	-21.415	-2.640
Time[30] * Phase[Butorphanol]	2.500	4.500	20.000	0.560	0.585	-6.888	11.888
Time[40] * Phase[Butorphanol]	-10.417	4.500	20.000	-2.310	0.031	-19.804	-1.029

Qt (mL minute^{-1} kg^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	66.172	5.958	5.000	11.110	0.000	50.857	81.486
Phase[Butorphanol]	-11.864	1.680	5.000	-7.060	0.001	-16.184	-7.545
Time[30]	13.070	3.076	20.000	4.250	0.000	6.654	19.486

Table 6-2, continued

Time[40]	-3.613	3.076	20.000	-1.170	0.254	-10.029	2.802
Time[30] * Phase[Butorphanol]	9.673	3.076	20.000	3.140	0.005	3.257	16.088
Time[40] * Phase[Butorphanol]	-5.311	3.076	20.000	-1.730	0.100	-11.726	1.105

PVR (mmHg * minute L^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	-1.193	0.073	5.000	-16.31	<.0001	-1.381	-1.005
Phase[Butorphanol]	0.174	0.063	5.000	2.740	0.041	0.011	0.336
Time[30]	-0.069	0.092	20.000	-0.750	0.462	-0.260	0.122
Time[40]	0.051	0.092	20.000	0.560	0.583	-0.140	0.242
Time[30] * Phase[Butorphanol]	-0.038	0.092	20.000	-0.410	0.684	-0.229	0.153
Time[40] * Phase[Butorphanol]	0.073	0.092	20.000	0.800	0.435	-0.118	0.264

f_H (beats $minute^{-1}$)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	105.389	4.166	5.000	25.300	<.0001	94.679	116.099
Phase[Butorphanol]	-14.167	4.739	5.000	-2.990	0.031	-26.348	-1.985
Time[30]	15.528	2.162	20.000	7.180	<.0001	11.018	20.037

Table 6-2, continued

Time[40]	-3.556	2.162	20.000	-1.640	0.116	-8.065	0.954
Time[30] * Phase[Butorphanol]	13.917	2.162	20.000	6.440	<.0001	9.407	18.426
Time[40] * Phase[Butorphanol]	-5.167	2.162	20.000	-2.390	0.027	-9.676	-0.657

SV (mL beat^{-1} kg^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	-0.501	0.086	5.000	-5.800	0.002	-0.724	-0.279
Phase[Butorphanol]	-0.057	0.040	5.000	-1.430	0.212	-0.158	0.045
Time[30]	0.052	0.054	20.000	0.960	0.347	-0.061	0.166
Time[40]	-0.029	0.054	20.000	-0.530	0.603	-0.142	0.085
Time[30] * Phase[Butorphanol]	0.035	0.054	20.000	0.650	0.526	-0.078	0.149
Time[40] * Phase[Butorphanol]	-0.034	0.054	20.000	-0.630	0.539	-0.148	0.079

T (PA, °C)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	37.917	0.228	5.000	166.66	<.0001	37.332	38.501
Phase[Butorphanol]	-0.367	0.104	5.000	-3.540	0.017	-0.633	-0.100
Time[30]	0.250	0.041	20.000	6.160	<.0001	0.165	0.335

Table 6-2, continued

Time[40]	-0.175	0.041	20.000	-4.310	0.000	-0.260	-0.090
Time[30] * Phase[Butorphanol]	0.167	0.041	20.000	4.110	0.001	0.082	0.251
Time[40] * Phase[Butorphanol]	-0.142	0.041	20.000	-3.490	0.002	-0.226	-0.057

T (rectal, °C)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	38.311	0.238	5.000	160.95	<.0001	37.699	38.923
Phase[Butorphanol]	-0.261	0.102	5.000	-2.560	0.051	-0.523	0.001
Time[30]	0.022	0.035	20.000	0.630	0.536	-0.051	0.096
Time[40]	0.106	0.035	20.000	2.990	0.007	0.032	0.179
Time[30] * Phase[Butorphanol]	0.128	0.035	20.000	3.620	0.002	0.054	0.201
Time[40] * Phase[Butorphanol]	0.061	0.035	20.000	1.730	0.099	-0.013	0.135

PaO_2 (mmHg)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	36.244	0.564	5.000	64.260	<.0001	34.795	37.694
Phase[Butorphanol]	5.956	0.692	5.000	8.600	0.000	4.176	7.735
Time[30]	-7.186	0.581	20.000	-12.36	<.0001	-8.399	-5.974

Table 6-2, continued

Time[40]	3.681	0.581	20.000	6.330	<.0001	2.468	4.893
Time[30] * Phase[Butorphanol]	-6.014	0.581	20.000	-10.35	<.0001	-7.226	-4.801
Time[40] * Phase[Butorphanol]	3.653	0.581	20.000	6.280	<.0001	2.440	4.865

$PaCO_2$ (mmHg)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	83.228	2.475	5.000	33.620	<.0001	76.865	89.591
Phase[Butorphanol]	-7.472	2.093	5.000	-3.570	0.016	-12.854	-2.091
Time[30]	8.414	0.979	20.000	8.590	<.0001	6.371	10.456
Time[40]	-3.894	0.979	20.000	-3.980	0.001	-5.937	-1.852
Time[30] * Phase[Butorphanol]	7.464	0.979	20.000	7.620	<.0001	5.421	9.506
Time[40] * Phase[Butorphanol]	-6.094	0.979	20.000	-6.220	<.0001	-8.137	-4.052

$P\bar{v}O_2$ (mmHg)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	25.631	0.977	5.000	26.230	<.0001	23.119	28.143
Phase[Butorphanol]	3.953	0.452	5.000	8.740	0.000	2.790	5.115
Time[30]	-5.389	0.429	20.000	-12.56	<.0001	-6.284	-4.494

Table 6-2, continued

Time[40]	2.686	0.429	20.000	6.260	<.0001	1.791	3.581
Time[30] * Phase[Butorphanol]	-3.494	0.429	20.000	-8.140	<.0001	-4.389	-2.599
Time[40] * Phase[Butorphanol]	2.681	0.429	20.000	6.250	<.0001	1.786	3.576

P_A-aO_2 (mmHg)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	3.195	0.098	5.000	32.510	<.0001	2.943	3.448
Phase[Butorphanol]	0.080	0.083	5.000	0.970	0.378	-0.133	0.294
Time[30]	-0.038	0.049	20.000	-0.770	0.452	-0.141	0.065
Time[40]	-0.007	0.049	20.000	-0.150	0.881	-0.110	0.095
Time[30] * Phase[Butorphanol]	-0.052	0.049	20.000	-1.060	0.302	-0.155	0.051
Time[40] * Phase[Butorphanol]	0.116	0.049	20.000	2.340	0.030	0.013	0.219

CaO_2 (mL dL^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	13.139	0.938	5.000	14.010	<.0001	10.728	15.550
Phase[Butorphanol]	2.581	0.380	5.000	6.800	0.001	1.605	3.557
Time[30]	-3.227	0.281	20.000	-11.50	<.0001	-3.812	-2.641

Table 6-2, continued

Time[40]	1.515	0.281	20.000	5.400	<.0001	0.930	2.100
Time[30] * Phase[Butorphanol]	-2.330	0.281	20.000	-8.310	<.0001	-2.915	-1.745
Time[40] * Phase[Butorphanol]	1.650	0.281	20.000	5.880	<.0001	1.065	2.235

$C\bar{v}O_2$ (mL dL^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	8.395	0.852	5.000	9.850	0.000	6.204	10.585
Phase[Butorphanol]	2.759	0.354	5.000	7.780	0.001	1.847	3.670
Time[30]	-3.208	0.254	20.000	-12.62	<.0001	-3.738	-2.678
Time[40]	1.692	0.254	20.000	6.660	<.0001	1.162	2.222
Time[30] * Phase[Butorphanol]	-2.352	0.254	20.000	-9.250	<.0001	-2.882	-1.822
Time[40] * Phase[Butorphanol]	1.741	0.254	20.000	6.850	<.0001	1.211	2.272

VEBTPS (mL $minute^{-1}$ kg^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	98.449	4.922	5.000	20.000	<.0001	85.797	111.102
Phase[Butorphanol]	3.011	4.248	5.000	0.710	0.510	-7.909	13.931
Time[30]	-0.127	2.877	20.000	-0.040	0.965	-6.128	5.874

Table 6-2, continued

Time[40]	5.190	2.877	20.000	1.800	0.086	-0.812	11.191
Time[30] * Phase[Butorphanol]	-4.769	2.877	20.000	-1.660	0.113	-10.770	1.233
Time[40] * Phase[Butorphanol]	5.261	2.877	20.000	1.830	0.082	-0.740	11.263

f_R (breaths minute^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	1.969	0.078	5.000	25.330	<.0001	1.769	2.169
Phase[Butorphanol]	0.167	0.055	5.000	3.030	0.029	0.025	0.308
Time[30]	-0.261	0.052	20.000	-5.040	<.0001	-0.369	-0.153
Time[40]	0.107	0.052	20.000	2.060	0.053	-0.001	0.215
Time[30] * Phase[Butorphanol]	-0.134	0.052	20.000	-2.590	0.018	-0.242	-0.026
Time[40] * Phase[Butorphanol]	0.134	0.052	20.000	2.580	0.018	0.026	0.242

V_T (mL breath^{-1} kg^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	14.669	1.175	5.000	12.480	<.0001	11.649	17.690
Phase[Butorphanol]	-2.096	0.945	5.000	-2.220	0.077	-4.525	0.334
Time[30]	4.067	0.905	20.000	4.490	0.000	2.180	5.955

Table 6-2, continued

Time[40]	-1.262	0.905	20.000	-1.390	0.178	-3.150	0.626
Time[30] * Phase[Butorphanol]	1.272	0.905	20.000	1.410	0.175	-0.615	3.160
Time[40] * Phase[Butorphanol]	-1.165	0.905	20.000	-1.290	0.213	-3.053	0.722

V_{DPHYS} (mL minute^{-1} kg^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	49.713	3.130	5.000	15.880	<.0001	41.668	57.757
Phase[Butorphanol]	-0.435	2.739	5.000	-0.160	0.880	-7.476	6.606
Time[30]	-1.223	1.661	20.000	-0.740	0.470	-4.688	2.243
Time[40]	1.684	1.661	20.000	1.010	0.323	-1.781	5.150
Time[30] * Phase[Butorphanol]	-0.800	1.661	20.000	-0.480	0.635	-4.266	2.665
Time[40] * Phase[Butorphanol]	1.295	1.661	20.000	0.780	0.445	-2.171	4.760

VO_2 (mL minute^{-1} kg^{-1})

Intercept	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Phase[Butorphanol]	4.392	0.151	5.000	29.040	<.0001	4.003	4.780
Time[30]	-0.513	0.171	5.000	-3.010	0.030	-0.952	-0.075
Time[40]	0.463	0.114	20.000	4.060	0.001	0.225	0.701

Table 6-2, continued

Time[30] * Phase[Butorphanol]	-0.718	0.114	20.000	-6.290	<.0001	-0.956	-0.480
Time[40] * Phase[Butorphanol]	0.533	0.114	20.000	4.670	0.000	0.295	0.771

VCO_2 (mL minute^{-1} kg^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	4.197	0.204	5.000	20.530	<.0001	3.671	4.722
Phase[Butorphanol]	-0.021	0.219	5.000	-0.100	0.927	-0.583	0.541
Time[30]	-0.098	0.137	20.000	-0.710	0.486	-0.384	0.189
Time[40]	0.080	0.137	20.000	0.580	0.567	-0.207	0.367
Time[30] * Phase[Butorphanol]	-0.036	0.137	20.000	-0.260	0.794	-0.323	0.250
Time[40] * Phase[Butorphanol]	0.369	0.137	20.000	2.690	0.014	0.083	0.656

DO_2 (mL minute^{-1} kg^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	7.830	0.636	5.000	12.310	<.0001	6.195	9.464
Phase[Butorphanol]	-0.058	0.246	5.000	-0.230	0.825	-0.691	0.576
Time[30]	-0.461	0.419	20.000	-1.100	0.285	-1.335	0.414
Time[40]	0.525	0.419	20.000	1.250	0.225	-0.349	1.399

Table 6-2, continued

Time[30] * Phase[Butorphanol]	0.128	0.419	20.000	0.310	0.763	-0.746	1.003
Time[40] * Phase[Butorphanol]	0.289	0.419	20.000	0.690	0.498	-0.585	1.163

OER (%)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	4.033	0.081	5.000	49.560	<.0001	3.824	4.242
Phase[Butorphanol]	-0.118	0.064	5.000	-1.860	0.123	-0.282	0.046
Time[30]	0.167	0.061	20.000	2.750	0.012	0.040	0.294
Time[40]	-0.234	0.061	20.000	-3.840	0.001	-0.361	-0.107
Time[30] * Phase[Butorphanol]	0.117	0.061	20.000	1.920	0.070	-0.010	0.244
Time[40] * Phase[Butorphanol]	-0.141	0.061	20.000	-2.320	0.031	-0.268	-0.014

Qs/Qt (%)

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	48.136	1.111	5.000	43.350	<.0001	45.282	50.991
Phase[Butorphanol]	-9.382	0.718	5.000	-13.07	<.0001	-11.228	-7.537
Time[30]	11.097	2.471	20.000	4.490	0.000	5.943	16.251
Time[40]	-6.202	2.471	20.000	-2.510	0.021	-11.356	-1.049

Table 6-2, continued

Time[30] * Phase[Butorphanol]	8.515	2.471	20.000	3.450	0.003	3.362	13.669
Time[40] * Phase[Butorphanol]	-3.206	2.471	20.000	-1.300	0.209	-8.359	1.948

Noradrenaline (ng mL^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	5.363	0.118	5.000	45.260	<.0001	5.058	5.667
Phase[Butorphanol]	0.002	0.050	5.000	0.040	0.973	-0.127	0.130
Time[30]	0.066	0.016	20.000	4.060	0.001	0.032	0.099
Time[40]	0.002	0.016	20.000	0.110	0.913	-0.032	0.035
Time[30] * Phase[Butorphanol]	0.017	0.016	20.000	1.080	0.294	-0.016	0.051
Time[40] * Phase[Butorphanol]	0.000	0.016	20.000	-0.020	0.982	-0.034	0.033

Tremor score

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	2.056	0.093	5.000	22.110	<.0001	1.817	2.295
Phase[Butorphanol]	-0.222	0.102	5.000	-2.170	0.082	-0.486	0.041
Time[30]	0.694	0.066	20.000	10.560	<.0001	0.557	0.832
Time[40]	-0.306	0.066	20.000	-4.650	0.000	-0.443	-0.168

Table 6-2, continued

Time[30] * Phase[Butorphanol]	0.139	0.066	20.000	2.110	0.047	0.002	0.276
Time[40] * Phase[Butorphanol]	-0.028	0.066	20.000	-0.420	0.677	-0.165	0.109

Lactate (mmol L^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	0.318	0.203	5.000	1.570	0.178	-0.203	0.839
Phase[Butorphanol]	-0.041	0.170	5.000	-0.240	0.820	-0.477	0.395
Time[30]	0.091	0.033	20.000	2.730	0.013	0.021	0.160
Time[40]	0.009	0.033	20.000	0.260	0.798	-0.061	0.078
Time[30] * Phase[Butorphanol]	-0.048	0.033	20.000	-1.440	0.166	-0.117	0.022
Time[40] * Phase[Butorphanol]	0.020	0.033	20.000	0.590	0.560	-0.050	0.089

Hb (g dL^{-1})

Term	Estimate	SE	DFDen	t Ratio	Prob > |t|	Lower 95%	Upper 95%
Intercept	15.422	0.234	5.000	65.830	<.0001	14.820	16.024
Phase[Butorphanol]	0.144	0.142	5.000	1.010	0.357	-0.221	0.510
Time[30]	0.394	0.093	20.000	4.240	0.000	0.200	0.589
Time[40]	-0.039	0.093	20.000	-0.420	0.681	-0.233	0.155

Table 6-2, continued

Time[30] * Phase[Butorphanol]	0.056	0.093	20.000	0.600	0.558	-0.139	0.250
Time[40] * Phase[Butorphanol]	0.139	0.093	20.000	1.490	0.152	-0.055	0.333

SD, standard deviation; mPAP, mean pulmonary arterial pressure; PAOP, pulmonary arterial occlusion pressure; mSAP, mean systemic arterial pressure; Qt, cardiac output; PVR, pulmonary vascular resistance; f_H, heart rate; SV, stroke volume; T, temperature; PA, pulmonary artery; PaO_2, arterial oxygen partial pressure; $PaCO_2$, arterial carbon dioxide partial pressure; $P\bar{v}O_2$, mixed venous oxygen partial pressure; $P_{A}\text{-}aO_2$, alveolar-arterial oxygen partial pressure difference; CaO_2, arterial oxygen content; $C\bar{v}O_2$, mixed venous oxygen content; V_{EBTPS}, minute ventilation, body temperature and pressure, saturated with water vapour; f_R, respiratory rate; V_T, tidal volume; V_{DPHYS}, physiological dead space ventilation; VO_2, oxygen consumption; VCO_2, carbon dioxide production; DO_2, oxygen delivery; OER, oxygen extraction ratio; Qs/Qt, shunt fraction; Hb, haemoglobin; SE, standard error; DFDen, degrees of freedom, denominator.

[a] Mixed venous lactate.

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APPENDIX: ETHICAL CONSIDERATIONS

During a pilot study, one rhinoceros received etorphine only by dart and became immobilised in sternal recumbency at 11.5 minutes post-administration. All monitoring equipment was attached during the 30 minute instrumentation period, except the Swan-Ganz catheter which could not be placed in the pulmonary artery (because the introducer could not be placed into the linguofacial vein). At approximately 31 minutes post-induction the rhinoceros appeared to be arousing; 300 mg of ketamine was administered intravenously (IV) to deepen the level of central nervous system (CNS) depression. In retrospect, the observed movement may have been agonal ventilation. The rhinoceros suffered cardiorespiratory arrest and, despite cardiopulmonary resuscitation efforts, including external chest compressions, oxygen supplementation, and initially, butorphanol, followed by multiple doses of naltrexone, adrenaline, and doxapram, by the investigators (including three board-certified anesthesiologists and four wildlife veterinarians), it died. Because gross post-mortem examination did not reveal any pathology that could explain the arrest, the cause of death was deemed likely to be hypoxaemia. A second rhinoceros ingested a foreign object (a small wire) and developed signs of esophageal obstruction (‘choke’) similar to horses. The rhinoceros was immobilised for examination, which revealed rupture and abscessation of the cervical esophagus, necessitating euthanasia. A third rhinoceros was excluded from the study because it scored ‘6’ on the VWS immobilisation scoring system (Table 3-3, Table 4-3) during two immobilisation attempts and required urgent administration of the opioid antagonist, naltrexone, before data collection could be completed. This left a sample size of six rhinoceros for data collection and analysis.

Immobilisation is crucial for managing the white rhinoceros population in southern Africa. The tragic death during immobilisation, and the rhinoceros that responded so poorly to immobilisation that data acquisition was abandoned, confirm the great risk associated with

immobilising white rhinoceros. Identifying the specific factors that contribute to this risk for mortality, and investigating possible preventive measures, requires systematic investigation. This, in turn, evidently requires putting some individuals at risk. That said, the rhinoceros chosen for this study were all sub-adult males; the rationale for this choice was that, apart from being a reasonably healthy and genetically homogenous population, this group is also considered to be of lesser importance to the general gene pool of white rhinoceros.

To develop and manage a system of national parks that represents the biodiversity, landscapes, and associated heritage assets of South Africa for the sustainable use and benefit of all.

South African NATIONAL PARKS

ANIMAL USE AND CARE COMMITTEE: APPLICATION FOR APPROVAL

A. PROJECT DETAILS

Project Title	"CARDIOPULMONARY EFFECTS OF ETORPHINE-BASED DRUG COMBINATIONS IN BOMA-HABITUATED VS. FREE-RANGING WHITE RHINOCEROS (*Ceratotherium simum*)"		
Researcher	Dr J. M. Boosch	SANParks Reference No.	001/16

B. SCIENTIFIC REVIEW STATEMENT

(Every application should be supported by a declaration that it has undergone prior scientific review through at least one of the SANParks Research Nodes.)

This research protocol has been reviewed by the Savanna and / or Arid Research Centres SANParks and has been judged to be of national importance, designed in accordance with accepted scientific practices and norms and is in the opinion of the reviewers likely to be successful in achieving its objective.

Name: [illegible] Designation: Acting ME Signature: [signature] Date: 10/3/2016

*As per SANParks Research Approval process

Note: In accordance with the South African National Standard (SANS 10386-2008): "The Care and Use of Animals for Scientific Purposes", an animal is regarded as being "live, sentient non-human vertebrate, including eggs, foetuses and embryos, that is, fish, amphibians, reptiles, birds and mammals, including domestic animals, purpose-bred animals, farm animals, wildlife and higher invertebrates such as advanced members from the Cephalopoda and Decapoda".

This form should be submitted with the SANParks standard Research Project Application, and (where relevant) the following supporting documents: CVs of practitioners in support of competence to handle or treat animals, notices of approval of other ethics committees, diagrams or references illustrating the equipment and/or techniques to be applied.

For Administrative Purposes			
Submission Date	9th Dec 2015	APPROVED	DISAPPROVED
AUCC approval / Disapproval Date	10/3/2016	Signature [signature]	
Reason for Decision	The study fully meets ethical standards		

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Cardiopulmonary effects of etorphine-based drug combinations in boma-habituated vs. free-ranging white rhinoceros *(Ceratotherium simum)***
PROJECT NUMBER	**V101-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**JM Boesch**

STUDENT NUMBER (where applicable)	**Not UP**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**White rhinoceros (Ceratotherium simum)**	
NUMBER OF ANIMALS	**28**	
Approval period to use animals for research/testing purposes		**November 2015-November 2016**
SUPERVISOR	**Dr. L Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	30 November 2015
CHAIRMAN: UP Animal Ethics Committee	Signature	

S*4285-15*

UNIVERSITEIT VAN PRETORIA
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Animal Ethics Committee

Extension No. 1

PROJECT TITLE	**Cardiopulmonary effects of etorphine-based drug combinations in boma-habituated vs. free-ranging white rhinoceros *(Ceratotherium simum)***
PROJECT NUMBER	**V101-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**JM Boesch**

STUDENT NUMBER (where applicable)	**Not UP**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**White rhinoceros *(Ceratotherium simum)***	
NUMBER OF ANIMALS	**28**	
Approval period to use animals for research/testing purposes		**November 2016-November 2017**
SUPERVISOR	**Dr. LCR Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 22 November 2016
CHAIRMAN: UP Animal Ethics Committee	Signature

S***4285-15***

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

Extension No. 2

PROJECT TITLE	**Cardiopulmonary effects of etorphine-based drug combinations in boma-habituated vs. free-ranging white rhinoceros *(Ceratotherium simum)***
PROJECT NUMBER	**V101-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**JM Boesch**

STUDENT NUMBER (where applicable)	**Not UP**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**White rhinoceros *(Ceratotherium simum)***	
NUMBER OF ANIMALS	**28 approved; 8 used**	
Approval period to use animals for research/testing purposes		**February 2018-February 2019**
SUPERVISOR	**Dr. LCR Meyer**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 8 February 2018
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15