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REDUCTION OF CAPTURE-INDUCED HYPERTHERMIA AND RESPIRATORY DEPRESSION IN UNGULATES

Leith Carl Rodney Meyer

A thesis submitted to the Faculty of Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Doctor of Philosophy

Johannesburg, South Africa, 2009

DECLARATION

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

I declare that the work contained in this thesis is my own, unless otherwise acknowledged.

This work has not been submitted before for any degree or examination at any other university.

_____

Leith Meyer

Signed on the _____ day of _____, 2009

ABSTRACT

When wild animals are captured they often develop capture-related side-effects that may result in morbidity and mortality. During chemical capture of wild ungulates, capture-induced hyperthermia and opioid-induced respiratory depression occur commonly. Little is known about the mechanisms of capture-induced hyperthermia, and the effects of opioid drugs on respiratory function still need to be clarified. Also, current methods of reversing opioid-induced respiratory depression are inadequate. I therefore investigated the mechanisms and patterns of capture-induced hyperthermia, by continuously measuring body temperatures of impala during different capture procedures. I also investigated the effects of opioid drugs on respiratory function and pulmonary performance by examining the changes of cardiorespiratory variables before and during opioid immobilization of goats and impala. Concurrently, I investigated whether serotonergic ligands could be used to reverse the opioid-induced respiratory depression that occurred in these animals.

I found stress to be the major factor associated with capture-induced hyperthermia, with exercise playing a minor role. I also found that environmental thermal conditions and the pharmacological effects of the capture drugs played no role in inducing capture-induced hyperthermia. I found that the opioid drug etorphine, which is commonly used to chemically capture wild animals, not only causes depression in respiratory rhythm and ventilation, but also a decrease in alveolar-arterial oxygen exchange. I demonstrated that serotonergic ligands with agonist effects at 5-HT_{1A} and 5-HT_4 receptors partially reversed opioid-induced respiratory depression and hypoxia, predominantly by improving alveolar-arterial oxygen exchange, presumably by

increasing pulmonary perfusion and improving ventilation perfusion ratios, but also, in some cases, by improving ventilation.

I advise that to limit the morbidity and mortality associated with capture-induced hyperthermia, procedures that cause the least stress should be used and animals should be exposed to stressors for the shortest time possible. The use of anxiolytic drugs to reduce stress may also be considered. If animals are captured by chemical immobilization with opioid drugs their respiratory function should be monitored closely. Counting breaths does not adequately monitor respiratory function and methods to assess carbon dioxide and oxygen levels in arterial blood should be used. If respiratory depression occurs, efforts to reverse this depression should not focus only on improving ventilation but also should aim at improving gas exchange in the lungs. Serotonergic ligands with agonist effects at 5-HT_{1A} and 5-HT_4 receptors could be used to achieve these aims. Although some of these ligands can cause arousal in immobilized animals, if they are administered with the opioid they enhance the induction of catatonic-immobilization, and their use in a dart may not only improve knock down times, thereby minimising stress and capture-induced hyperthermia, but they may also prevent opioid-induced respiratory depression.

ACKNOWLEDGMENTS

I am indebted to many people who assisted me in this challenging and enlightening endeavour. Firstly, I would like to thank my supervisors and friends, Andrea Fuller and Duncan Mitchell, who both acted as mentors to me, who believed in my ideas, patiently taught and guided me, and allowed me the freedom to explore this field of research. I would also like to thank many colleagues from the School of Physiology who helped me collected data: Linda Fick and Robyn Hetem who supported me in almost all of my data collection trials, Philippa Hidden and Marna Broekman for helping me to establish the protocol for testing serotonergic ligands in the field in impala, Dr Peter Kamerman, Dr Alida Faurie, Brenda de Witt, Georg von Bormann, Lennox Nqobo and the late David Makoa, for their dedicated support and assistance with experimental procedures. I also would like to thank many other colleagues and students, too numerous to name, whose contributions however small, were significant, both in the field and in the laboratory.

I thank the National Zoological Gardens for allowing us access to the use of the facilities at the Lichtenburg Game Breeding Centre. I am most grateful to the staff from the Wits Central Animal Service and Lichtenburg Game Breeding Centre who cared for my animals and who dedicated many hours of animal handling and husbandry. In particular, I thank the former head of the Game Breeding Centre, André Matthee, for his friendship and unfailing support for my field research. I thank Novartis Animal Health for their donation of some etorphine and diprenorphine, Geoff Salter for developing a processor for monitoring blood pressures and Susan Dantu from Arrow Africa for a donation of intra-arterial catheters.

Last, but not least, I thank my family; my wife, Philippa, for her calm but enduring support and her patience through the arduous times. To Charlie, my daughter, to watch you grow and develop has brought great joy and encouragement to me as I've made the finishing touches to this thesis.

The work in this thesis was funded by the National Research Foundation, South Africa, and grants from the Medical Faculty Research Endowment Fund, and the University Research Committee, University of the Witwatersrand.

TABLE OF CONTENTS

		Page
DECLARATION …….....		II
ABSTRACT …….		III
ACKNOWLEDGEMENTS …….		V
TABLE OF CONTENTS …….		VII
LIST OF FIGURES …….		X
LIST OF TABLES ……...		XIV
AUTHOR CONTRIBUTIONS ……		XV
LIST OF ABBREVIATIONS ……..		XIX
CHAPTER 1		
Introduction ……		1
1.1	Capture-induced hyperthermia ……	3
1.1.1	Introduction …….	3
1.1.2	Possible mechanisms involved in capture-induced hyperthermia ….....	3
1.2	Opioid-induced respiratory depression ……...	18
1.2.1	Introduction …….	18
1.2.2	Control of respiration and the mechanisms that are involved in opioid-induced respiratory depression ……	21
1.2.3	Reduction of opioid-induced respiratory depression ……..	27

1.3 The consequences of capture-induced hyperthermia and opioid-induced respiratory depression ……. 40

1.4 Thesis aims …….. 47

CHAPTER 2

Hyperthermia in captured impala (*Aepyceros melampus*): A fright not flight response. Journal of Wildlife Diseases 44, 404-416, 2008 ……..... 52

CHAPTER 3

Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilization with 4 different drug combinations. Journal of the South African Veterinary Association 79, 121-129, 2008 ……..... 66

CHAPTER 4

Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats. American Journal of Physiology - Regulatory Integrative and Comparative 290, R405-R413, 2006 …… 76

CHAPTER 5

Effects of serotonin agonists and doxapram on respiratory depression and hypoxemia in etorphine-immobilized impala (*Aepyceros melampu).*

In press: Journal of Wildlife Diseases ……. 86

CHAPTER 6

Conclusion …….. 114

6.1 Reduction of capture-induced hyperthermia ……... 125

6.2 Opioid-induced respiratory depression and its reversal ……. 123

6.3 Recommendations and conclusions ……... 125

REFERENCES …… 132

LIST OF FIGURES

CHAPTER 1

Fig. 1 Effects of diazepam and aspirin on stress-induced hyperthermia in mice …….. 13

Fig. 2 Respiratory control centres in the brainstem …….. 22

Fig. 3 Signal transduction pathways of the 5-$HT_{4(a)}$ and μ-opioid receptors. 33

Fig. 4 Signal transduction pathways of the 5-HT_{1A}, 5-HT_7 and μ-opioid receptors ……. 34

Fig. 5 Signal transduction pathways of the 5-HT_{2A}, 5-HT_{2B} and μ-opioid receptors ……. 36

CHAPTER 2

Fig. 1 Body temperature, measured at 10-min intervals in one, naïve (non-habituated) female impala over 13 days, in which capture occurred on day six ……... 57

Fig. 2 Mean body temperatures, measured at 10-min intervals, of six naïve impala before, during and after immobilization by darting with etorphine and fentanyl, in random order, on two separate non-consecutive days …… 58

Fig. 3 Body temperatures, measured at 10-min intervals, of two naïve (non-habituated) impala on one day, in which they were chased and caught in capture nets ……..... 58

Fig. 4 Body temperatures, measured at 10-min intervals, of two impala immobilized successively, about 30 min apart, in a group of five animals that were habituated for four months …… 58

Fig. 5 Change in body temperature, time to recumbency after darting and cortisol concentration in four groups of immobilized impala …… 59

Fig. 6 Change in body temperature in six 'naïve impala' and five 'four-month habituated impala' in response to disturbance when other animals in the group were darted and chemically immobilized ……. 60

Fig. 7 Change in body temperature, in response to darting and chemical immobilization, of 15 impala which were habituated for two and five months and then darted with etorphine and azaperone, *versus* the mean dry bulb temperature and mean black globe temperature ... 61

CHAPTER 3

Fig. 1 Body temperatures (mean ± SD, *n* =15), measured at 10-min intervals, of impala before, during and after immobilization with etorphine and azaperone, etorphine and medetomidine, thiafentanil and azaperone, and thiafentanil and medetomidine ……... 69

Fig. 2 Change in body temperature, 4-h thermal response index and time to recumbency after darting impala …… 70

Fig. 3 Change in body temperature *vs.* time to recumbency, plasma cortisol concentration *vs.* time to recumbency and haemoglobin oxygen saturation over the first four min of immobilization *vs.* time to recumbency …… 71

Fig. 4 Peak body temperature after darting *vs.* body temperature at 20 min after darting and 30 min after darting …… 72

Fig. 5 Change in body temperature *vs.* dry bulb temperature and 4-h thermal response index *vs.* dry bulb temperature ……... 73

Fig. 6 Respiratory rate, haemoglobin oxygen saturation and heart rate in chemically immobilized impala ……. 74

CHAPTER 4

Fig. 1 Drug effects on respiratory rates over time …… 79

Fig. 2 Drug effects on percentage haemoglobin saturation of arterial blood by oxygen ……... 79

Fig. 3 Drug effects on arterial partial pressure of oxygen ……. 80

Fig. 4 Drug effects on arterial partial pressure of carbon dioxide …… 80

Fig. 5 Drug effects on the alveolar arterial oxygen partial pressure gradient 81

Fig. 6 Drug effects on heart rates …….. 81

Fig. 7 Drug effects on mean arterial pressure ……... 82

CHAPTER 5

Fig. 1 Change in arterial partial pressure of oxygen, arterial partial pressure of carbon dioxide and alveolar arterial oxygen partial pressure gradient, in impala immobilized with etorphine, in response to injection I.V. of injectable water, doxapram, metoclopramide and buspirone …….. 101

Fig. 2 Change in respiratory rate, minute ventilation and tidal volume, in impala immobilized with etorphine, in response to injection I.V. with injectable water, doxapram, metoclopramide and buspirone ……. 102

Fig. 3 Change in the percentage of arterial haemoglobin oxygen saturation and median movements, in impala immobilized with etorphine, in response to injection I.V. with injectable water, doxapram, metoclopramide and buspirone …….. 103

Fig. 4 Change in arterial partial pressure of oxygen, respiratory rate and minute ventilation, in impala immobilized with etorphine, in response to injection I.V. with injectable water, DMSO and pimozide ……. 104

LIST OF TABLES

CHAPTER 1

Table 1. Summary of the potential causes of heat production that may result in capture-induced hyperthermia ..……. 16

Table 2. Summary of opioid drugs used to chemically capture wildlife ….. 20

Table 3. Serotonergic and dopaminergic ligands investigated for reversal of opioid-induced respiratory depression …….. 28

CHAPTER 2

Table 2. Summary of differences in immobilization regimens, activity levels and habituation between groups ……. 55

CHAPTER 5

Table 1. Agents used during the experimental trials …… 92

Table 2. Description used to score the movements of the impala during immobilization ……... 95

AUTHOR CONTRIBUTIONS

Chapter 2:

Meyer LCR, Fick LG, Matthee A, Mitchell D and Fuller A (2008) Hyperthermia in captured impala (*Aepyceros melampus*): A fright not flight response. Journal of Wildlife Diseases 44, 404-416.

The idea for this study arose from discussions with my co-authors. I prepared the equipment, performed the surgical procedures, collected, analyzed and interpreted the data, and wrote the manuscript. A small amount of the data in this paper was collected from another study in which I was not involved but my co-authors were. However, I analyzed and interpreted all the data. Linda Fick assisted with the planning and execution of the experimental work, and edited the manuscript. André Matthee led the capture procedures and assisted with the experimental work and animal husbandry. Duncan Mitchell and Andrea Fuller, my supervisors, helped with planning of the study, experiments and interpretation of the data, and edited the manuscript.

Chapter 3:

Meyer LCR, Hetem RS, Fick LG, Matthee A, Mitchell D and Fuller A (2008) Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilization with 4 different drug combinations. Journal of the South African Veterinary Association 79, 121-129.

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The idea for this study was my own. I prepared the equipment, collected, analyzed and interpreted the data, and wrote the manuscript. Linda Fick and Robyn Hetem assisted with some of the experimental work, and edited the manuscript. Duncan

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

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

Andrea Fuller: _____

Date: _____

Duncan Mitchell: _____

Date: _____

LIST OF ABBREVIATIONS

α	-	alpha
A	-	adenosine
A-a gradients	-	alveolar-arterial oxygen partial pressure gradient
ATP	-	adenosine triphosphate
β	-	beta
BIMU-8	-	*N*-[(1*R*,5*S*)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl]-2-oxo-3-(propan-2-yl)-2,3-dihydro-1*H*-benzimidazole-1-carboxamide hydrochloride
BW 723C86	-	5-((thiophen-2-yl)methoxy)-α-methyltryptamine
C	-	degrees Celsius
°C	-	degrees Celsius
cAMP	-	adenosine 3',5'-monophosphate
cc	-	millilitre
CO_2	-	carbon dioxide
δ	-	delta
D	-	dopamine
DMSO	-	dimethyl sulphoxide
E	-	east
FiO_2	-	fractional inspired oxygen
GABA	-	gamma aminobutyric acid
Gi/o	-	inhibitory hetero-trimeric guanine-nucleotide-binding protein
Gs	-	stimulating hetero-trimeric guanine-nucleotide-binding protein
h	-	hour

ha	-	hectare
I.M.	-	intramuscular
I.V.	-	intravenous
κ	-	kappa
kg	-	kilogram
km	-	kilometre
kPa	-	kilopascal
m	-	meter
mg	-	milligram
min	-	minute
ml	-	millilitre
mm	-	millimetre
mmHg	-	millimeters mercury
mo	-	month
n	-	number
O_2	-	oxygen
$PaCO_2$	-	arterial partial pressure of carbon dioxide
PAO_2	-	alveolar partial pressure of oxygen
PaO_2	-	arterial partial pressure of oxygen
Pb	-	barometric pressure
PH_2O	-	water vapour pressure
PO_2	-	partial pressure of oxygen
RER	-	respiratory exchange ratio
S	-	south
SaO_2	-	arterial haemoglobin oxygen saturation

SD	-	standard deviation
S.C.	-	subcutaneous
SNK	-	Student Neuman Keuls
t/a	-	trading as
Tb	-	body temperature
µ	-	mu
µg	-	microgram
wk	-	week
yr	-	year
5-HT	-	serotonin
6-chloro APB	-	6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine
8-OH-DPAT	-	8-hydroxy-2-dipropylaminotetralin hydrobromide
%	-	percent

CHAPTER 1

Introduction

The conservation of wildlife is important not only for maintaining the earth's rich biodiversity, but also for the economies of many countries, as eco-tourism generates substantial revenue. An essential component of wildlife conservation and management is the capture and transport of wild animals. Techniques for capturing wild animals have improved substantially over the past few decades, reducing both animal mortalities and the costs of capture (McKenzie 1993). However, an unacceptable number of animals still die or experience morbidity as a result of capture-related complications; mortalities as high as 43% have been reported in some capture operations (Knottenbelt 1990; Knox & Hattingh 1992; Murray et al. 1981).

Any attempt at capturing a wild animal causes arousal in that animal (Knox 1992). That arousal induces the release of stress hormones (Hattingh et al. 1990), which changes the physiology of the animal's body, preparing it for potential danger (Knox & Hattingh 1992; Meltzer & Kock 2006b). When animals are unable to escape the perceived danger, this arousal leads to an extreme psychological and physiological response which may result in stress, trauma, hyperthermia, capture myopathy, shock and ultimately death (Meltzer & Kock 2006b). To reduce arousal and the related adverse events, wild animals usually are captured by chemical immobilization with potent drug combinations. While these drugs sedate and calm animals, they also compromise the animal's normal physiological function and may lead to bloat, vomiting or regurgitation, respiratory depression, hyperthermia, cardiovascular depression, shock and death (Meltzer & Kock 2006b).

In this thesis I will address two of the adverse events most commonly associated with mortality during wildlife capture, namely capture-induced hyperthermia and opioid-

induced respiratory depression. This introductory chapter reviews the current knowledge of the mechanisms that result in, the consequences of, and the possible ways of reducing these side-effects, and poses the questions that are addressed in the thesis.

1.1 Capture-induced hyperthermia

1.1.1 Introduction

Wild animals typically develop an acute hyperthermia when captured (Cheney & Hattingh 1987; Fuller et al. 2005; Gericke et al. 1978; Hofmeyr et al. 1973; Jessen et al. 1994; Kock et al. 1987a; Kock et al. 1987b; Martucci et al. 1992; Montané et al. 2003; Montané et al. 2007). Although body temperature sometimes is measured and documented during capture operations, very little is said about the cause, significance and consequences of its elevation. Nevertheless, it has been proposed that capture-induced hyperthermia may play a significant role in capture myopathy and acute death (Antognini et al. 1996; Cheney & Hattingh 1987; Gericke et al. 1978; Meltzer & Kock 2006b). However, the mechanisms involved in inducing this hyperthermia are not well understood and methods of preventing and treating capture-induced hyperthermia are based on intuition and anecdotal information, with little or no systematic investigation of whether they are beneficial.

1.1.2 Possible mechanisms involved in capture-induced hyperthermia

For hyperthermia to develop in an animal its heat production needs to exceed its heat loss. When this situation occurs heat is stored in the body and the magnitude of this heat storage is determined by the addition of the metabolic heat production to the rate

of heat transfer via evaporative, convective, radiative and conductive means between the animal and the environment (Jessen 2001; Willmer et al. 2005).

Metabolic heat production increases via a number of mechanisms. An increase in resting metabolic rate can be achieved by increasing the metabolic activity of body organs and is usually brought about by either an increase in thyroid hormone activity or an increase in nutrient intake (Jansky 1995; Jessen 2001). The increase in resting metabolic rate is not usually associated with a sudden increase in heat production but rather with a slow increase over the long-term (Jessen 2001). Capture-induced hyperthermia, on the other hand, is associated with an acute precipitous increase in heat production and body temperature (Fuller et al. 2005; Jessen et al. 1994) and therefore it is unlikely that an elevation in resting metabolic rate via thyroid hormones and nutrient intake is involved.

Acute increases in metabolic heat production can be brought about by other metabolic processes. These include shivering and non-shivering thermogenesis. Non-shivering thermogenesis occurs predominantly in brown adipose tissue and is brought about by the activation of sympathetic fibres which increases the rate of fatty acid oxidation via uncoupling proteins in the mitochondria (Jessen 2001; Willmer et al. 2005). This oxidative process bypasses the production of ATP and therefore energy released from this oxidation is not conserved, as would normally occur, but rather is dissipated into the body as heat (Jessen 2001). Although this process is highly effective at producing body heat, in some rodent species it can increase up to 500% of resting heat production (Guyton & Hall 1996), its role during capture-induced hyperthermia, particularly in large adult mammals, is still questionable. The reason is that the

general accepted dogma states that in larger species the presence of brown adipose tissue is restricted mainly to the perinatal period (Jessen 2001), with very few brown fat cells occurring in white fat tissue in adult life (Jansky & Jansky 2002). However, with the development of fluorodeoxyglucose positron emission tomography (FDG-PET) significant amounts of brown adipose tissue have been indentified in adult humans (Nedergaard et al. 2007; Virtanen et al. 2009) and, this tissue appears to play a significant role in the control of body temperature and adiposity (Saito et al. 2009). However, these studies have only been done in humans and therefore the occurrence and metabolic significance of brown adipose tissue in other large adult mammals still needs to be determined. Shivering, on the other hand, may play a role in capture-induced hyperthermia. Shivering is an involuntary tremor of the skeletal muscle that is used primarily to defend normal body temperature during cold exposure (Jessen 2001; Willmer et al. 2005). Two phases of shivering occur, the first being associated with continuous trains of action potentials to single motor units that result in an increase in muscle tension, and the second being associated with group discharges of motor units that result in oscillatory muscle movements (Jessen 2001). Although animals are usually normothermic before capture, and therefore not shivering, I have observed both muscle tension and oscillatory muscle movements in some animals during capture. Whether muscle tension and overt shivering always occur during capture and whether the changes are brought about by a thermal stimulus, or are centrally regulated in reaction to stress, needs to be determined.

Acute increases in metabolic heat production also can be brought about by increasing energy turnover in the organs of the body. During capture, animals are often, but not always, highly active as they try to escape being captured (Burroughs & McKenzie

1993; Meltzer & Kock 2006b). This activity increases the work done by the skeletal muscles and also increases heat production of the muscles; up to 75% of the nutrient energy utilized during muscle contraction is released in the form of heat (Guyton & Hall 1997; Schönbaum & Lomax 1991d). The heat produced by muscle contraction during capture is not only limited to that of the skeletal muscles but also is produced by the heart and respiratory muscles as the activity of these muscles increases as the body's demand for oxygen increases (Jessen 2001). Although animals often engage in high-intensity exercise during capture, when they are relatively inactive or in confinement during capture, they also display acute increases in body temperatures (Bakken et al. 1999). Therefore, unlike the current assumptions of game capture personnel that activity is the sole cause of capture-induced hyperthermia, activity does not appear to be essential for its development.

An increase in heat production in animals can also be brought about by derangements in metabolism. Porcine Stress Syndrome is one of these derangements, where a precipitous rise in body temperature, severe lactacidosis, muscle pathology and acute death with the rapid onset of rigor are induced by natural stressors such as transport, high ambient temperature, exercise, fighting, copulation and parturition (Patterson & Allen 1972). Although this syndrome is described in pigs, many other species including humans, horses, zebra and a number of antelope and bird species develop the same clinical symptoms after extreme bouts of exercise. Because of these similar symptoms Porcine Stress Syndrome has been equated to the cause of exertional myopathy in man and horses, and capture myopathy in wild animals (Mitchell & Heffron 1982). However, the distinguishing feature of Porcine Stress Syndrome, compared to exertional myopathy and capture myopathy, is that pigs that develop

Porcine Stress Syndrome are stress-susceptible and develop aberrant metabolic changes even after exposure to minor stressors. In addition, these pigs have a genetic deficit which also predisposes them to develop drug-induced malignant hyperthermia (Mitchell & Heffron 1980).

Drug-induced malignant hyperthermia is a hereditary membrane disorder which is brought about mainly by anaesthetic agents and results in a hypercatabolic derangement in metabolism (Ali et al. 2003; Schönbaum & Lomax 1991a). The pathophysiology of malignant hyperthermia involves an uncontrolled release of cytoplasmic free calcium from the sarcoplasmic reticulum which leads to the activation of energy-liberating biochemical pathways and results in a precipitous rise in body temperature, hyperkalaemia and acidosis (Ali et al. 2003). Malignant hyperthermia has been reported in humans, pigs, dogs, cats, horses and cattle (Schönbaum & Lomax 1991a). It is induced predominantly by halogenated volatile anaesthetics like halothane, isoflurane and enflurane, and depolarizing muscle relaxants like succinylcholine (Ali et al. 2003); other drugs like local anaesthetics and caffeine also may trigger its occurrence (Schönbaum & Lomax 1991a). Drug-induced malignant hyperthermia has been shown to play no role in the development of capture myopathy (Antognini et al. 1996) and no reports have been documented during wildlife capture. Indeed, the drugs that induce malignant hyperthermia are used rarely in wildlife capture, with the exception of succinylcholine which is used to capture crocodiles and cull elephants (Burroughs et al. 2006), both of which don’t show malignant hyperthermia. Therefore a role for drug-induced malignant hyperthermia in capture-induced hyperthermia is unlikely.

However, it is possible that drugs that are used in capture may cause other derangements in metabolism that increase heat production. Neuroleptic drugs, like azaperone, haloperidol, zuclopenthixol acetate and perphenazine enanthate are used extensively as tranquilizers during capture and the housing of wildlife (Meltzer & Kock 2006a; Swan 1993a). These drugs may cause neuroleptic malignant syndrome, a syndrome that has been documented mainly in humans and is associated with a drug-induced hyperthermia. Its pathogenesis is unlike that of drug-induced malignant hyperthermia but it causes a similar hypermetabolic state (Nisijima et al. 2007; Ohnishi 1994). Although there is no documented evidence, it is possible that neuroleptic malignant syndrome may contribute to capture-induced hyperthermia, but only when neuroleptic drugs are used; neuroleptics are not always used during capture. In addition, neuroleptics affect thermoregulation through several mechanisms, including anti-adrenergic effects, inhibition of sweating, and central dopamine antagonism, which disrupt the thermoregulatory set-point causing thermal lability (Fick 2007; Fick et al. 2006). This disruption in thermoregulation would alter the rate of heat transfer between the animal and the environment and, under high environmental heat loads, also may contribute to an increase in an animal's body temperature during capture.

A drug-induced disruption in thermoregulation is not only limited to the neuroleptic drugs but also may occur with the sedatives and immobilizing agents that are used during capture. α_2-agonists are drugs that are used commonly as sedatives during chemical capture. These drugs induce thermal lability (Jalanka & Roeken 1990; Maskrey et al. 1970) by affecting the central noradrenergic mechanisms that control body temperature regulation (Cremer & Bligh 1969). The opioids, which are used as

immobilizing agents in the majority of herbivore species, also alter thermoregulation. Their effects on thermoregulation are complex and depend on either the drug, the dose, the route of administration, the species, ambient conditions and the behavioural state of an animal (Schönbaum & Lomax 1991c). Meltzer and Kock (2006b) proposed that during chemical capture the adrenergic effects of opioid drugs increase metabolic rate and, hence, heat production. Although the adrenergic effects of opioid drugs have been demonstrated in rats given etorphine (Roquebert & Delgoulet 1988), these effects have not been linked to body temperature changes, so the effect of the opioid drugs on capture-induced hyperthermia is unclear.

Apart from the possible role of capture drugs in capture-induced hyperthermia, it has also been proposed that environmental conditions prevailing during capture influence body temperatures (Ebedes et al. 1996; McKenzie 1993). From these proposals, it has become accepted in the game capture industry that animals should not be caught when ambient temperatures exceed 25˚C, and therefore that capture operations should be restricted to the early mornings and late afternoons (Ebedes et al. 1996; Kock et al. 2006; McKenzie 1993). The primary objective of these recommendations is to ensure that hyperthermic animals are able to dissipate body heat to the environment after capture (Meltzer & Kock 2006b). However, these recommendations also have led to the assumption that animals will become hyperthermic if they are captured on hot days (Ebedes et al. 1996; Wenger et al. 2007). This assumption may well be true if capture-induced hyperthermia was a passive hyperthermia brought on by high environmental heat loads, but it is unlikely as many animals that are captured on cold days also develop capture-induced hyperthermia (Montané et al. 2003; Montané et al. 2007).

An increase in metabolic heat production during capture can also be brought about by changes in circulating hormones. A number of hormones such as adrenaline, noradrenaline, cortisol, glucagon and insulin enhance the activity of metabolic pathways and therefore are thermogenic (Jansky 1995; Schönbaum & Lomax 1991d). However, the role of hormonal thermogenesis in capture-induced hyperthermia is not known. What is known is that capture causes the release of some of these hormones, but their conventional role is believed to be related to a reaction to stress rather than to thermogenesis (Hattingh & Petty 1992).

Finally, stress, itself, may stimulate an increase in heat production and play a role in the generation of capture-induced hyperthermia. Although it has been proposed that stress plays a role in capture-induced hyperthermia (Burroughs & McKenzie 1993; Meltzer & Kock 2006b; Montané et al. 2003; Montané et al. 2007), especially in captures that result in capture myopathy (Gericke et al. 1978; Harthoorn 1976; Mitchell & Heffron 1982), it is uncertain how important its role is. Although stress is difficult to quantify, its occurrence can be identified by measuring the body's behavioural, biochemical and cellular reactions after exposure to a stressor (Hattingh 1988b; Möstl & Palme 2002). By using these measures, the occurrence of stress has been identified in wild animals in different capture procedures and in a wide variety of species (Cheney & Hattingh 1987; Ganhao et al. 1989; Ganhao et al. 1988; Hattingh 1988a; Hattingh & Petty 1992; Knox & Hattingh 1992; Knox & Zeller 1993; Kock et al. 1987a; Kock et al. 1987b; Martucci et al. 1992; Morton et al. 1995; Read et al. 2000). Acute stress results in a centrally mediated response, through the activation of the hypothalamic-pituitary-adrenal axis and the sympatho-adrenal-

medullary system (Henry 1992), which alters an animal's physiology in preparation for a fight or flight response (Meltzer & Kock 2006b).

Activation of the hypothalamic-pituitary-adrenal axis results in the enhanced secretion of corticotropin releasing hormone, adrenocorticotrophic hormone and glucocorticoids (Guyton & Hall 1997). Corticotropin releasing hormone has thermogenic effects, but these effects are dependent on sympathetic activation of brown adipose tissue (Olivier et al. 2003; Rothwell 1990). The thermogenic effects of glucocorticoids also appear to be dependent on the metabolic activation of brown adipose tissue (Hampl et al. 2006). Thus it is unlikely that glucocorticoids and corticotropin releasing hormone increase body temperature of large adult animals during capture. On the other hand, the activation of the sympatho-adrenal-medullary system with the subsequent release of adrenaline and nor-adrenaline (Guyton & Hall 1997) may increase the body temperature of animals during capture. Normal sympathetic stimulation and circulating adrenaline and nor-adrenaline decreases heat loss from the body by causing vasoconstriction and, it also increases the rate of cellular metabolism, but this increase usually increases the rate of heat production by only 10 to 15% (Guyton & Hall 1996). This effect is important during cold exposure in both small and large mammals as it helps maintain normal body temperature (Jansky & Jansky 2002). However, the infusion of high doses of exogenous catecholamines can increase metabolic rate by as much as 45% of basal metabolic rate in adult humans and sheep (Graham & Christopherson 1981; Lesna et al. 1999). Whether high surges of endogenous catecholamines, particularly those that occur during capture (Hattingh et al. 1988), can produce a similar effect is not known. Even if it could, this may not increase body temperature because Graham & Christopherson

(1981) showed that even though high doses of exogenous catecholamines increased sheep's metabolic rate by 45% it did not significantly increase their body temperature.

The rise in body temperature that is induced by stress is termed "stress-induced hyperthermia". Stress-induced hyperthermia is mediated by the autonomic nervous system and occurs prior to and during exposure to stressors like noise, heat, handling, novelty or pain (Olivier et al. 2003). Stress-induced hyperthermia occurs concurrently with the endocrine stress response, which is brought about by the stimulation of the hypothalamic-pituitary-adrenal axis and the sympatho-adreno-medullary system (Groenink et al. 1994). However, it does not appear that this hyperthermia results from the direct effect of hypothalamic-pituitary-adrenal axis stimulation, because stress-induced hyperthermia can be reduced even while the hypothalamic-pituitary-adrenal axis remains activated (Olivier et al. 2003). Because of these and other experimental findings it has been suggested that stress-induced hyperthermia is a psychogenic fever that is centrally regulated and brought about by an elevation in the thermoregulatory "set point", similar to that of fever (Briese & Cabanac 1991). This mechanism was proposed because cyclooxygenase inhibitors, which attenuate fever, have been shown to partially attenuate stress-induced hyperthermia (Michel et al. 2003; Morimoto et al. 1991). However, the view that stress-induced hyperthermia is a psychogenic fever has been strongly contested and, recently, Vinkers *et al.* (2009) have shown unequivocally (see example in Fig. 1) that stress-induced hyperthermia and infection-induced fever are two distinct processes mediated largely by different neurobiological mechanisms. However, their findings still cannot explain why cyclooxygenase inhibitors partially attenuate the rise in body

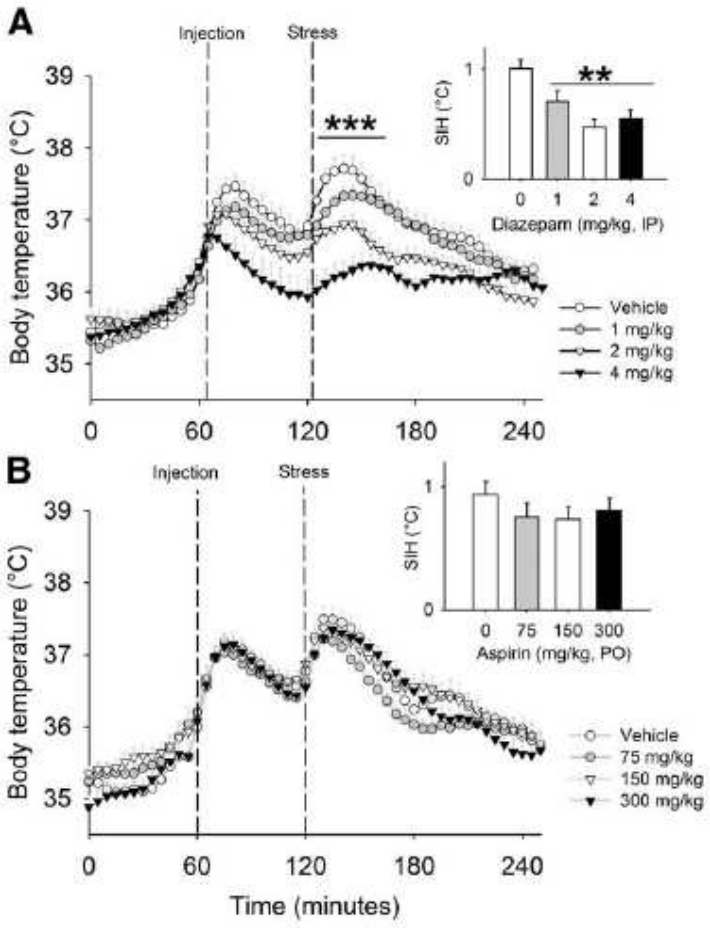


Figure 1. Effects of (A) diazepam (0-4mg.kg^{-1}, IP) and (B) aspirin (0-300mg.kg^{-1}, PO) on stress-induced hyperthermia in C57 mice (n=11). Note the stress-induced hyperthermia induced by the stress of handling at injection (60 min), and that of being placed in a new cage (120 min). The anxiolytic diazepam attenuates the stress-induced hyperthermia but the cyclooxygenase inhibitor aspirin does not. **: p<0.01, ***: p<0.001. (Taken from Figure 1. Vinkers CH, Groenink L, van Bogaert MJV, Westphal KGC, CJ Kalkman CJ, van Oorschot R, Oosting RS, Olivier B, and Korte SM (2009) Physiology & Behavior 98: 37-43.)

temperature in some models of stress in rats. Oka *et al.* (2001) propose that stress-induced hyperthermia, or as they term it "psychological stress-induced rise in core temperature" can be induced by two different mechanisms, a prostaglandin E_2-dependent mechanism (psychogenic fever) and a prostaglandin E_2-independent mechanism (anticipatory anxiety-induced rise in core temperature). Their proposal explains why cyclooxygenase inhibitors partially attenuate the rise in body temperature in some models of stress like "open-field stress", but not in others like "anticipatory or anxiety stress" (Oka et al. 2001). However, any model of psychological stress will induce some form of anxiety and therefore it seems plausible that the main cause of the rise in body temperature, irrespective of which stress model is used, will be because of prostaglandin E_2-independent mechanisms.

It has been proposed that stress-induced hyperthermia is ubiquitous because it is present in virtually any mammal that has been tested so far (Bouwknecht et al. 2007; Vinkers et al. 2008). In rats and mice it has been studied extensively and its occurrence in different stress models has allowed it to be used as a measure of stress and anxiety in behavioural and pharmacological studies (Bouwknecht et al. 2007; Olivier et al. 2003; Vinkers et al. 2008). It has been speculated that stress-induced hyperthermia is a component of capture-induced hyperthermia in wild animals (Bakken et al. 1999; Burroughs & McKenzie 1993; Montané et al. 2003; Montané et al. 2007), however, no studies have validated this theory. Although stress-induced hyperthermia has been the subject of many studies it is still unclear as to how it is mediated (Oka et al. 2001) and, like all the other potential causes of hyperthermia, it is uncertain as to what role it may play in capture-induced hyperthermia.

Several different mechanisms may be involved in capture-induced hyperthermia, but few have been tested (see Table 1 for a summary of the potential causes of capture-induced hyperthermia). To successfully prevent or treat capture-induced hyperthermia it is essential to understand how it is caused and the mechanisms involved in the generation or retention of body heat. Systematic studies using different capture procedures which involve the use of capture drugs, capture without drugs, capture with varying environmental conditions and capture with varying levels of exercise and stress, are required to elucidate these mechanisms.

Table 1. Summary of the potential causes of heat production that may result in capture-induced hyperthermia

Potential cause of heat production	Likelihood	Tested	Reference
Non-shivering thermogenesis	Possible – In humans there is growing evidence that metabolically active brown adipose tissue occurs in adult life	Untested – requires clarification of the occurrence and metabolic significance of brown adipose tissue in other large adult mammalian species	(Nedergaard et al. 2007; Saito et al. 2009; Virtanen et al. 2009)
Shivering	Possible – muscle tension and shivering have been observed during capture	Untested	(Jessen 2001; Willmer et al. 2005)
Exercise metabolism	Possible – exercise will increase body temperature but capture-induced hyperthermia can occur in inactive animals	Untested	(Bakken et al. 1999)
Drug-induced malignant hyperthermia	Unlikely – capture-induced hyperthermia occurs irrespective of whether drugs are used or not	Tested	(Antognini et al. 1996)
Neuroleptic malignant syndrome	Unlikely - capture-induced hyperthermia occurs irrespective of whether neuroleptic drugs are used or not	Untested	(Nisijima et al. 2007; Ohnishi 1994)

Potential cause of heat	Likelihood	Tested	Reference
Adrenergic effects of opioid drugs	Possible – opioid drugs have been shown to have adrenergic effects, but capture-induced hyperthermia occurs even when no opioids are used	Untested - requires clarification, there is no evidence that the adrenergic effects of the opioids cause hyperthermia	(Schönbaum & Lomax 1991b)
Environmental	Unlikely – capture hyperthermia can occur in cold environments	Untested	(Montané et al. 2003; Montané et al. 2007)
Hormonal metabolism	Possible – a number of hormones, particularly stress hormones are known to be thermogenic	Untested	(Jansky 1995)
Stress	Likely – stress-induced hyperthermia appears to be ubiquitous in mammals exposed to stressors	Untested	(Bouwknecht et al. 2007; Vinkers et al. 2008)

1.2 Opioid-induced respiratory depression

1.2.1 Introduction

The therapeutic uses of the opioids include the alleviation of pain and coughing, induction and maintenance of anaesthesia, antidiarrhoeal and chemical immobilization of wildlife (Bowdle 1988; Meltzer & Kock 2006a; Rang et al. 2003; Swan 1993b). However, their benefits can be produced at a cost to breathing and tissue oxygenation, which can lead to irreversible tissue damage and death (Bowdle 1988; Eilers & Schumacher 2004). These effects are always important as they may contribute significantly to mortality when wild animals are chemically captured (Haigh 1990; Meltzer & Kock 2006b).

The opioids that are used to chemically immobilize wildlife are derived from alkaloids found in opium or synthetic substances based on morphine's structure (Meltzer & Kock 2006b). These opioids are highly potent; etorphine has an analgesic potency up to 10000 times that of morphine (Swan 1993a). Because of these high potencies and their intrinsic activities, opioids that are used to chemically capture wildlife may cause respiratory depression. In some species, like canids and primates, opioids tend to cause severe central nervous system depression, while in others, like porcids, felids and equids, they tend to cause excitement (Hannon & Bossone 1991; Swan 1993b). Therefore, opioids are used to immobilize and capture only certain species which mostly come from the orders of perissodactyla, artiodactyla and proboscidae (see Table 2 for a summary of the opioid drugs commonly used to immobilize wildlife). Even within these species the opioids have varying effects, with some species being more prone to developing respiratory depression than others.

Those species that are more susceptible to respiratory depression include giraffe, hippopotamus, waterbuck, impala and white rhino (McKenzie 1993; Meltzer & Kock 2006b). There also are inherent differences to the pharmacological effects of the opioids amongst individuals within a species and thus some individuals may be more susceptible to developing respiratory depression than others (Ballanyi et al. 1997). However, when any animal is chemically captured from the wild, it is not possible to accurately predict its mass and therefore drug overdosing and adverse events like respiratory depression can occur in any species or individual irrespective of their susceptibilities. Therefore it is important to understand the effects of opioids on respiratory function and determine how to reverse opioid-induced respiratory depression, without reversing the immobilizing properties of the opioids, during chemical capture of wild animals.

Table 2. Summary of opioid drugs used to chemically capture wildlife (Kock et al. 2006; McKenzie 1993)

Opioid generic name	Opioid trade name and distributor	Species immobilized	Comments
Etorphine hydrochloride	M99, Norvatis, RSA or Captivon 98, Wildlife Pharmaceuticals, RSA	From the orders of perissodactyla, artiodactyla and proboscidae	Most commonly used opioid agonist in Southern Africa
Fentanyl	Sublimase, Janssen Animal Health, RSA	Mainly used in small artiodactyla, also used in canids e.g. wild dogs	Opioid agonist, 1/15 the potency of etorphine. Ineffective in equids
Carfentanil	Wildnil, Wildlife Pharmaceuticals, USA	As for etorphine	Opioid agonist Not available in Southern Africa More potent than etorphine, faster action and longer duration Not very effective in equids
Thiofentanil	A3080, Wildlife Pharmaceuticals, RSA	As for etorphine	Opioid agonist Potency similar to etorphine but faster action and shorter duration Ineffective in equids
Butorphanol tartrate	Formulated to obtain high enough concentrations	Experimental use in a number of species including the carnivores	Opioid agonist-antagonist Does not cause full immobilization Less respiratory depressant effects

1.2.2 Control of respiration and the mechanisms that are involved in opioid-induced respiratory depression

Breathing is controlled by a neuronal network that is found in the lower brainstem. This neuronal network innervates spinal motoneurones causing periodic contractions of respiratory muscles, which ventilate the lungs (Richter & Spyer 2001). During rapid shallow breathing contraction of respiratory muscles and the flow of air into and out of the lungs is generated by inspiratory and post-inspiratory (stage 1 expiration) neuronal phases. During deeper breathing these phases are supplemented by a third active expiratory (stage 2 expiration) phase (Ramirez & Richter 1996; Richter et al. 2000; Richter & Spyer 2001). The inspiratory neuronal phase corresponds to the contraction of inspiratory muscles, the post-inspiratory phase to passive exhalation where inspiratory muscles cease to contract and upper airway adductor muscles relax, and the active expiratory phase to the period where expiratory muscles contract (Bonham 1995; Haji et al. 2000).

The neuronal respiratory network in the brainstem is composed of groups of neurones that control and regulate the cycling of the respiratory phases (See Fig. 2). These groups of neurones are arranged bilaterally in the brainstem and consist of a dorsal and ventral respiratory group and pontine respiratory centres (Bonham 1995). The respiratory groups contain different types of respiratory neurones that discharge at different times throughout the respiratory cycle (Bonham 1995). Respiratory activity is controlled by synaptic interactions between the different types of respiratory neurones, and these interactions activate or inhibit the phases of the respiratory cycle to generate respiratory rhythm (Richter & Spyer 2001). The ventral

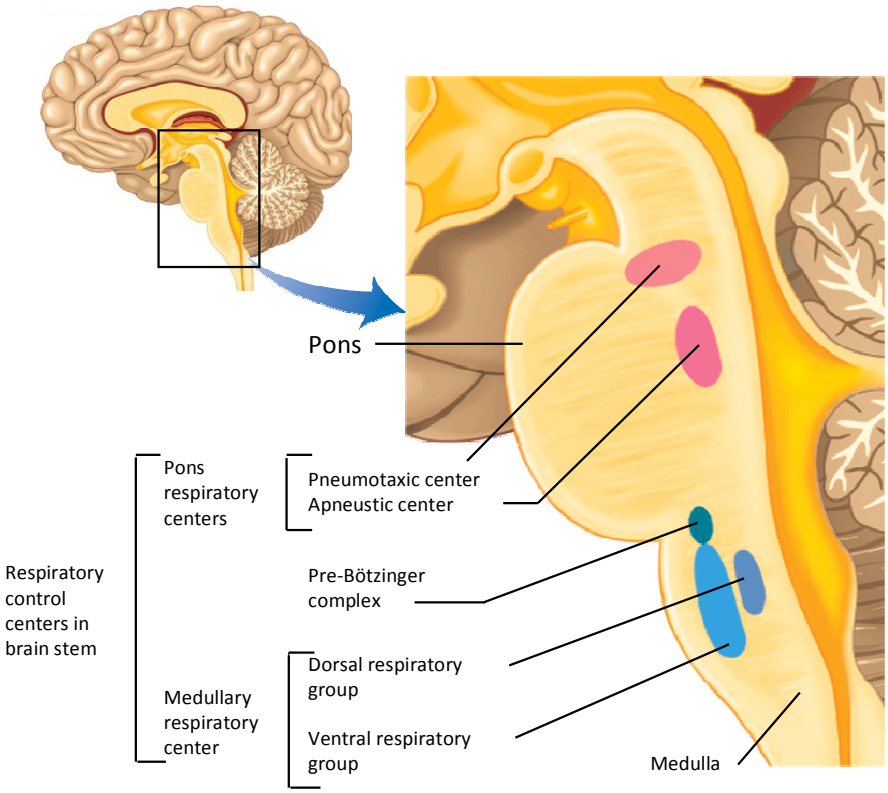


Figure 2. Respiratory control centres in the brainstem

(Taken from Figure 13-33. Sherwood L (2001, 4th Edition), Brooks/Cole Thomson Learning, Pacific Grove, USA, pp. 766)

respiratory group contains all of the different types of respiratory neurones and therefore is essential for rhythm generation (Bonham 1995; Richter & Spyer 2001). The ventral respiratory group has been divided into caudal, intermediate, rostral, pre-Bötzinger, and Bötzinger complexes and the parafacial group. Of these the pre-Bötzinger complex has been identified to be the main area that generates respiratory rhythm as it contains a kernel of respiratory neurones that have intrinsic bursting properties necessary for respiratory rhythm generation (Schwarzacher et al. 1995; Smith et al. 1991). However, there is currently debate about whether the pre-Bötzinger complex is the primary respiratory rhythm generator, or whether the parafacial respiratory group determines the rhythm of the neurones in the pre-Bötzinger complex (Onimaru et al. 2006). Despite this controversy it appears that the pre-Bötzinger complex plays a major role in respiratory rhythm generation (Onimaru et al. 2006; Peña & García 2006) and emerging evidence shows that the pre-Bötzinger complex and the parafacial respiratory group act as a coupled oscillator with modulating influences from the pons (Pattinson 2008).

Respiratory rhythm also is modulated by many sensory inputs that are external to the known respiratory neuronal groups. These inputs help regulate respiratory pattern generation and the volume and frequency of breathing (Gray et al. 1999; Ramirez & Richter 1996). The effects of these sensory inputs, and the connectivity of the neurones of the respiratory network, occur through a number of neurotransmitters that activate specific receptors. The principal neurotransmitters in the respiratory network are glutamate, GABA and glycine (Bonham 1995; Haji et al. 2000; Richter et al. 1997). Serotonin, catecholamines, acetylcholine, substance P, somatostatin, cholecystokinin, and endogenous opioids play a secondary neuromodulatory role

(Bonham 1995; Haji et al. 2000; Richter et al. 1997). The role of many of these neurotransmitters in respiratory control is well known, but it is not clear what role endogenous opioids play. It appears that they are not involved in the generation of normal respiration (Bonham 1995), but rather that they play a role during acute stress and in abnormal respiratory states like sleep apnoea and hypoxic apnoea. The role of endogenous opioids during these events has been proposed to be neuroprotective as they reduce excitotoxicity of neurones (Richter et al. 2000; Shook et al. 1990). Although endogenous opioids appear to play no role in normal respiration, opioid receptors are highly expressed throughout the respiratory network (Lonergan et al. 2003a; Pattinson 2008) and are particularly concentrated in the pre-Bötzinger complex (Gray et al. 1999; Manzke et al. 2003).

Opioid drugs cause respiratory depression mainly through their activation of opioid receptors that occur in respiratory neurones (McCrimmon & Alheid 2003; Shook et al. 1990). Different opioid drugs do not cause equivalent respiratory depressive effects as their effects on respiratory function are dependent on which opioid receptors they activate. The activation of mu(μ)-opioid receptors, in particular μ_2 receptors, causes respiratory depression (Haji et al. 2000; Shook et al. 1990). There is also evidence that activation of both delta (δ)- and kappa (κ)-opioid receptors also causes respiratory depression (Ballanyi et al. 1997; Bowdle 1988; Lonergan et al. 2003b; Takeda et al. 2001), but the main depressant effects of opioids appear to be caused by the activation of μ_2-opioid receptors (Haji et al. 2000).

The activation of opioid receptors results in a number of effects that cause respiratory depression. The neurones in the pre-Bötzinger complex appear to be the most

sensitive to μ-opioid agonists and therefore the most important of these effects is a disturbance in respiratory rhythm (Lalley 2003; Pattinson 2008). Opioids also disturb respiratory rhythm by activating respiratory neurones in the pons and also reduce the ability of the ventral respiratory group neurones to react to the chemoreceptor response to hypercapnia and hypoxia (Lalley 2008; McCrimmon & Alheid 2003; Pattinson 2008; Santiago & Edelman 1985; Shook et al. 1990). Opioids also depress the neurones that regulate tidal volumes, and thus may decrease the depth of breathing (Lalley 2003; Lalley 2008). They also inhibit the actions of vagal motorneurones which promote dilation of vocal folds (Lalley 2003), thereby causes an increase in upper airway resistance (Lalley 2008). In addition, opioids cause catatonia (Haveman & Kuschinsky 1982), which results in generalised muscle rigidity and a decrease in the chest wall compliance (Chen et al. 1996). They also increase the resistance to chest wall expansion by tonically activating expiratory neurones during inspiration (Lalley 2003; Lalley 2008). The respiratory depressant effects of the opioids are not limited to their effects on respiratory neurones in the central neuronal network. Opioids have a direct action on carotid bodies, which causes a decrease in chemoreceptor discharge and thus further depresses the response to hypoxia and hypercapnia (Pattinson 2008; Santiago & Edelman 1985). Opioids also activate pulmonary J receptors which may cause opioid-induced apnoea (Bowdle 1998; McKenzie 1993; Santiago & Edelman 1985; Shook et al. 1990). The combination of all these effects will lead to a decrease in respiratory pump action and cause hypoventilation with its associated pathophysiological effects.

The effects of the opioids, on both the central and peripheral neurones, therefore play a major role in decreasing pulmonary ventilation. However, the respiratory depressant

effects of the opioids are not limited to just the effects on ventilation; opioids also have marked effects on the cardiovascular system that may cause a decrease in the perfusion both of the lungs and other organs thus decreasing oxygen delivery to tissues. The effects of opioids on the cardiovascular system are complex and dependent on the opioid and dose used (Bowdle 1998). However, the opioids used during chemical immobilization of wildlife generally cause systemic and pulmonary vasoconstriction (Haigh 1990; Heard et al. 1990; Heard et al. 1996; Santiago & Edelman 1985) by either activating the sympathetic nervous system (Hannon & Bossone 1991; Heard et al. 1990; Heard et al. 1996; Roquebert & Delgoulet 1988) or possibly by causing the release of histamine (Hakim et al. 1992; Mather 1994). These vascular effects would result in a decrease in blood perfusion of body tissues, and would cause ventilation perfusion mismatching or shunting of blood in the lungs, and a decrease in oxygen diffusion across the alveolar membrane (Nunn 1993). Few studies have assessed pulmonary blood shunting and oxygen diffusion during opioid immobilization of animals. Two of the studies that have done so, have shown that opioid immobilization causes a decrease in oxygen diffusion in immobilized rhinoceros (Wenger et al. 2007) and elephant (Still et al. 1996). However, in another study in goats (Buss & Meltzer 2001) (Buss 2000), the authors argue that opioid immobilization did not result in pulmonary blood shunting. Their claims, however, appear to be erroneous as they calculated shunt fraction based on the incorrect assumption that the partial pressure of oxygen in the alveoli is equal to that of the partial pressure of oxygen in end capillary pulmonary vessels.

Although much is known about the neurological control of respiration, and the effects of opioids on this control, a better and more integrative understanding of the effects of

the opioids on cardio-respiratory control and function is needed, especially if more effective treatments for opioid-induced respiratory depression are to be established.

1.2.3 Reduction of opioid-induced respiratory depression

Many different pharmacological agents have been used in attempts to reduce or reverse the respiratory depressant effects of the opioids in both man and other animals (see table 3 for summary of receptor and respiratory activity of ligands recently investigated to reverse opioid-induced respiratory depression). Opioid receptor antagonists, or partial opioid receptor antagonists, like naloxone, nalorphine, butorphanol, nalbuphine, diprenophine and naltrexone have relieved respiratory depression successfully, but their major drawback is that they cause unwanted side-effects by blocking analgesia, causing cardiovascular instability, and reversing anaesthesia and chemical immobilization (Bowdle 1988; Swan 1993b). Analeptic drugs cause widespread central nervous system stimulation which results in increased ventilation (Bowdle 1988). Some of the analeptics like bemegride, nikethamide and picrotoxin, which have been used in the past, have lost favour because of their convulsant effects (Swan 1993b). One analeptic still used is doxapram. Unlike other

Table 3. Serotonergic and dopaminergic ligands investigated for reversal of opioid-induced respiratory depression

Ligand	Dose	Species	Respiratory effect	Known receptor activity		References
				Primary	Secondary	
BIMU-8	1-2 $mg.kg^{-1}$	Rats	increased phrenic nerve activity and respiratory minute volume	5-$HT_{4(a)}$ agonist	5-HT_3 antagonist	(Manzke 2004; Manzke et al. 2003; Wang et al. 2007)
Mosapride	15 mg	Humans	no effect on respiratory minute volume	5-HT_4 agonist		(Lötsch et al. 2005)
	100 $mg.kg^{-1}$	Rats				
8-OH-DPAT	10-100 $\mu g.kg^{-1}$	Rats	restored respiratory rate and increased respiratory minute volume	5-HT_{1A} agonist	5-HT_7 agonist	(Manzke 2004; Sahibzada et al. 2000; Wang et al. 2007)
Buspirone	50 $\mu g.kg^{-1}$	Rats	restored respiratory rate	5-HT_{1A} agonist	dopamine receptor dependant effects	(Sahibzada et al. 2000)
	60 mg	Humans	no effect on respiratory minute volume			

Ligand	Dose	Species	Respiratory effect	Known receptor activity		References
				Primary	Secondary	
α-methyl-5-HT	100 $\mu g.kg^{-1}$	Rat	no effect on respiratory neuronal activity	5-HT_{2A}		(Manzke 2004)
BW 723C86	1 $mg.kg^{-1}$	Rat	no effect on respiratory neuronal activity	5-HT_{2B}		(Manzke 2004)
6-chloro APB	3 $mg.kg^{-1}$	Cat	increased respiratory rate and depth and improved HbO_2 and $ETCO_2$	D_1-dopamine agonist		(Lalley 2005a)
Dihydrexidine	1 $mg.kg^{-1}$	Cat	increased respiratory rate and depth and improved HbO_2 and $ETCO_2$	D_1-dopamine agonist		(Lalley 2005a)

$ETCO_2$ – end tidal carbon dioxide, HbO_2 – Oxygen saturation of haemoglobin

analeptics, doxapram has a wide safety margin; its convulsant dose is 70-75 times the dose that stimulates respiratory activity (Swan 1993b). Doxapram not only causes central nervous system stimulation but it also stimulates peripheral chemoreceptors (Haji et al. 2000; Nishino et al. 1982; Swan 1993b). Its use, especially during chemical immobilization, is limited by its short duration of effect (Swan 1993b), its low concentration and efficacy after intramuscular injection which limits its use in a dart, and its side-effects, which include cortical arousal, tachycardia, hypertension, hyperthermia and muscle rigidity (Bowdle 1988). Due to the limitation of the current therapies, novel pharmacological agents are needed to reverse and prevent opioid-induced respiratory depression without causing unwanted side-effects, and preserving the therapeutic value of opioids, particularly when they are used as analgesic and immobilizing agents.

Improved understanding of the mechanisms involved in the generation and control of respiration allows for the investigation of potential novel pharmacological agents to reverse opioid-induced respiratory depression. Investigations have focused predominantly on neurotransmitters and other agents that activate pathways that alter the production of adenosine 3’,5’-monophosphate (cAMP). The rationale for this approach is that cAMP-dependent protein kinase activities increase neuronal activity which is particularly important for the generation and maintenance of stable respiratory rhythm (Ballanyi et al. 1997). Additionally, opioid receptor activation causes a decrease in cAMP; opioid receptors are coupled to Gi/o proteins which inhibit adenylyl cyclase, an enzyme essential for the production of cAMP (Ballanyi et al. 1997; Manzke et al. 2003).

Initial approaches to stimulate respiration focused on increasing cAMP by reducing its breakdown. Methylxanthines, like theophylline, aminophylline and caffeine, which block phosphodiesterase, the enzyme that catabolises cAMP, have some respiratory stimulant effects (Eldridge et al. 1983; Wilken et al. 2000). These respiratory effects are not due solely to their effects on phosphodiesterase but also to their antagonistic effects on adenosine A_1 and A_2 receptors (Rang et al. 2003). However, methylxanthines appear to be clinically useful only as bronchodialators and for the treatment of central, non-drug induced, apnoeas (Peña & García 2006; Rang et al. 2003; Wilken et al. 1997).

Recent steps at increasing respiratory neuronal cAMP have focussed on the activation of receptors that directly increase the activity of adenylyl cyclase. A promising advance has been the discovery that dopamine D_1-receptor activation can reverse opioid-induced neuronal respiratory depression (Lalley 2004; 2005b) through the activation of adenylyl cyclase (Ballanyi et al. 1997), without reversing opioid-induced analgesia (Lalley 2005a). However, at present there are no therapeutic D_1-receptor agonists that cross the blood brain barrier (Lalley 2005a). Therefore, reversing opioid-induced neuronal respiratory depression with D_1-receptor agonists in clinical situations currently is not feasible.

More promising steps have been made at increasing cAMP in respiratory neurones with serotonergic ligands that activate specific serotonin receptors in the neuronal network. In the brainstem medullary raphe neurones that produce serotonin project onto respiratory neurones (Richter et al. 2003). Although endogenous serotonin plays an important neuromodulatory role during normal breathing (Cao et al. 2006; Hodges & Richerson 2008; Richter et al. 2003), its

effects on respiratory neurones appear to be complex and are not clearly defined (Bonham 1995). Serotonin can have both a facilitatory and depressive effect on respiratory function (Sessle & Henry 1985), which can be attributed to its activation of multiple 5-HT-receptor subtypes found throughout the respiratory network (Peña & García 2006). The facilitatory effects of serotonin appear to be associated with an activation of serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_4 and 5-HT_7-receptors (Lalley et al. 1995; Manzke 2004; Peña & García 2006; Richter et al. 2003).

5-HT_4 receptors, particularly the splice variant 5-$HT_{4(a)}$, are highly expressed in respiratory neurones of the pre-Bötzinger complex (Manzke et al. 2003). Manzke et al. (2003) demonstrated that 5-$HT_{4(a)}$ and μ-opioid receptors were coexistent on these neurones and both were coupled to adenylyl cyclase in a antagonistic manner; μ-opioid receptors decrease cAMP through Gi proteins and 5-$HT_{4(a)}$ receptors increase cAMP through Gs proteins (Fig. 3). Similarly, 5-HT_7 receptors also are positively coupled to adenylyl cyclase (Vanhoenacker et al. 2000) and coexistent with opioid receptors on these neurones (Richter et al. 2003; Fig. 4). However, the expression of 5-HT_7 receptors in the pre-Bötzinger complex is relatively low (Richter et al. 2003). The coexistence of μ-opioid receptors and 5-$HT_{4(a)}$ and 5-HT_7 receptors on pre-Bötzinger complex respiratory neurones, and the contrasting action of the opioids and serotonin on adenylyl cyclase, has allowed for the use of serotonergic ligands with 5-$HT_{4(a)}$ and 5-HT_7 actions to reverse opioid-induced depression of respiratory neurones (Manzke et al. 2003; Richter et al. 2003; Wang et al. 2007).

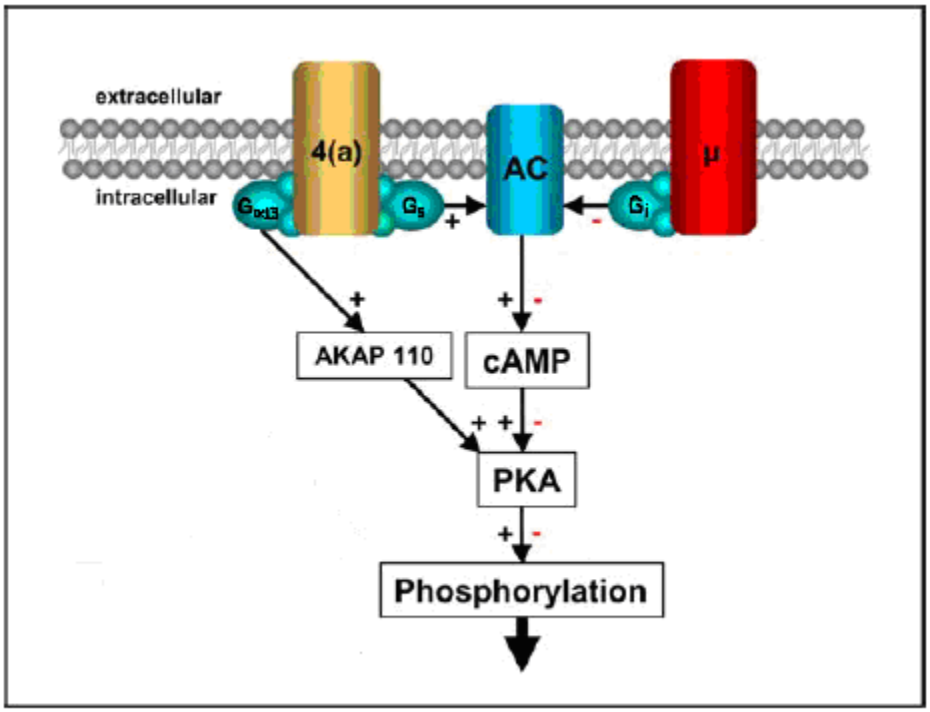


Figure 3. Signal transduction pathways of the 5-$HT_{4(a)}$ and μ-opioid receptors: abbreviations: adenylyl cyclase (AC), protein kinase (PKA), cyclic adenosine 5', 3'-monophosphate (cAMP), serotonin 4(a) receptor (4(a)), μ-opioid receptor (μ), stimulating or inhibitory hetero-trimeric guanine-nucleotide-binding protein (G_s or G_i), hetero-trimeric guanine-nucleotide-binding protein α13 ($G_{\alpha 13}$), A-kinase-anchoring protein 110 (AKAP 110) (Redrawn from Figure 71, Manzke T. Unpublished PhD thesis, University of Göttingen, Göttingen, Sweden).

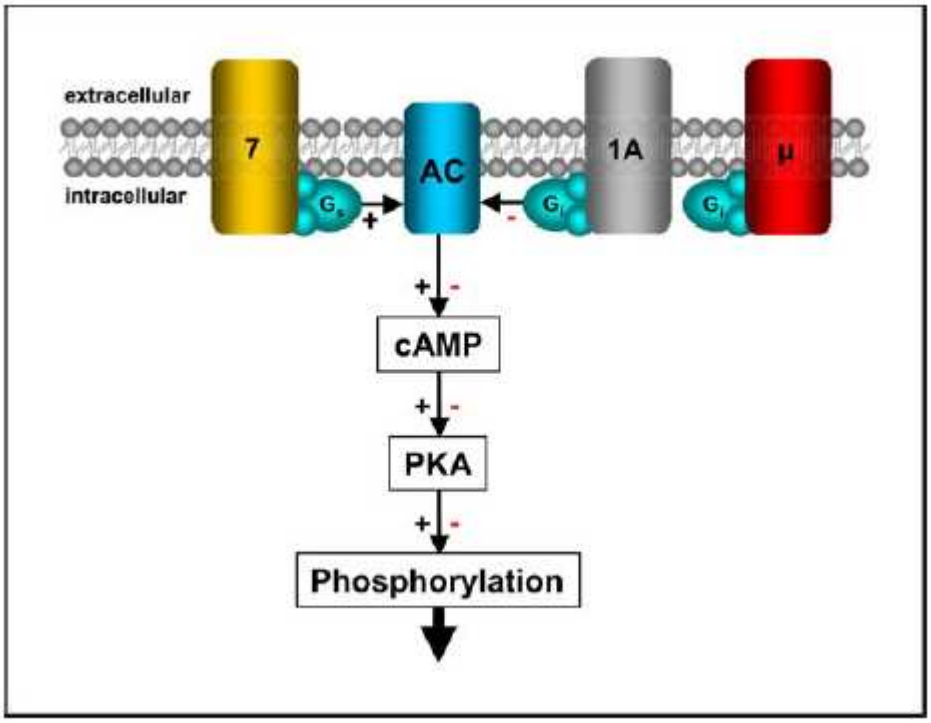


Figure 4. Signal transduction pathways of the 5-HT_{1A}, 5-HT_7 and μ-opioid receptors: abbreviations: adenylyl cyclase (AC), protein kinase (PKA), cyclic adenosine 5', 3'-monophosphate (cAMP), serotonin 7 receptor (7), serotonin 1A receptor (1A), μ-opioid receptor (μ), stimulating or inhibitory hetero-trimeric guanine-nucleotide-binding protein (G_s or G_i) (Taken from Figure 72, Manzke T. Unpublished PhD thesis, University of Göttingen, Göttingen, Sweden).

The possibilities of reversing opioid-induced neuronal respiratory depression with 5-HT_{2A}, 5-HT_{2B} and 5-HT_{1A} receptor agonists also have been studied. Although 5-HT_{2A} and 5-HT_{2B} receptors are expressed in the pre-Bötzinger complex and their activation in the absence of opioids increases respiratory activity (Lalley et al. 1995; Manzke 2004; Peña & Ramirez 2002), agonists of these receptors are not able to reverse opioid-induced neuronal respiratory depression (Manzke 2004). These findings could be explained by these receptors being coupled to phospholipase C, which activates respiratory activity via mechanisms that are distinct to those which are activated via adenylyl cyclase (Manzke 2004; Fig. 5). 5-HT_{1A} receptors, on the other hand, are coupled to adenylyl cyclase, but they inhibit adenylyl cyclase and decrease the production of cAMP (Richter et al. 2003; Fig. 4). However, agonists of 5-HT_{1A} receptors stimulate respiration (Garner et al. 1989; Mendelson et al. 1990) and reverse opioid-induced neuronal respiratory depression (Manzke 2004; Sahibzada et al. 2000; Wang et al. 2007). How 5-HT_{1A} agonists affect respiratory function and reverse opioid-induced neuronal respiratory depression is not understood, but possible mechanisms have been proposed. In the central respiratory network, 5-HT_{1A} receptors are the serotonin receptor subtype expressed most extensively and these receptors are involved in depressing respiratory neuronal excitability (Richter et al. 2003). Activation of post-synaptic 5-HT_{1A} receptors in pontine areas of the brainstem inhibits apneusis, which is a marked prolongation of inspiration, by inhibiting the activity of neurones that cause prolonged inspiratory effort (Barnes & Sharp 1999; Lalley et al. 1994a; Pierrefiche et al. 1998). This proposed mechanism couples with the proposed model by Manzke (2004) that suggests that 5-HT_{1A} agonists shift opioid-induced breathing patterns from a three-phase to a two-phase cycle by inhibiting early-inspiratory and post-inspiratory neurones, thus increasing respiratory rate. Although these proposed mechanisms have not been linked to

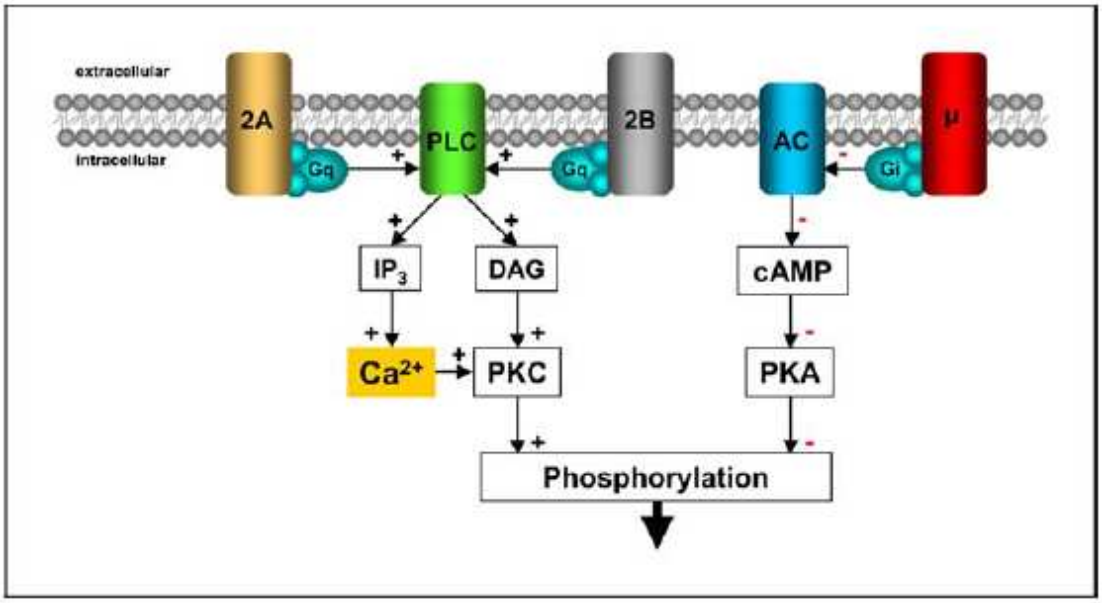


Figure 5. Signal transduction pathways of the 5-HT_{2A}, 5-HT_{2B} and μ-opioid receptors: abbreviations: serotonin 2A receptor (2A), serotonin 2B receptor (2B), phospholipase C (PLC), inositol 1,4, 5-triphosphate (IP_3), diacylglycerol (DAG), protein kinase C (PKC), hetero-trimeric guanine-nucleotide-binding protein q (Gq), adenylyl cyclase (AC), protein kinase (PKA), cyclic adenosine 5', 3'-monophosphate (cAMP), μ-opioid receptor (μ), inhibitory hetero-trimeric guanine-nucleotide-binding protein (G_i) (Taken from Figure 74, Manzke T. Unpublished PhD thesis, University of Göttingen, Göttingen, Sweden) .

the reversal of opioid-induced neuronal respiratory depression they may be plausible because the action of opioids on pontine neurones can cause apneustic respiratory arrhythmias (Lalley 2005c; Pattinson 2008).

Other mechanisms also may account for the ability of 5-HT_{1A} agonists to reverse opioid-induced neuronal depression. 5-HT_{1A} agonists are believed to reverse opioid-induced neuronal respiratory depression by increasing the excitability of respiratory motoneurones in the spinal cord (Teng et al. 2003). 5-HT_{1A} agonists also decrease the inhibitory effects of serotonin on respiratory neurones in the pre-Bötzinger complex by activating pre-synaptic 5-HT_{1A} autoreceptors in brainstem raphe neurones (Oertel et al. 2007). Activation of these autoreceptors leads to a decrease in opioid-induced neuronal serotonin release and dorsal raphe neuron activity, which results in antagonism of opioid-induced neuronal respiratory depression (Florez et al. 1972; Lalley et al. 1997; Tao & Auerbach 1995; Trulson & Arasteh 1986). It is possible that one, or a combination, of the above effects is involved in the reversal of opioid-induced respiratory neuronal depression by 5-HT_{1A} agonists, but further clarification is needed.

The effects of serotonergic ligands on the reversal of opioid-induced neuronal respiratory depression are not limited to the central nervous system but also involve haemodynamic effects that influence respiratory function. The most important of these haemodynamic effects is the reversal of opioid-induced pulmonary vasoconstriction (Haigh 1990; Heard et al. 1990; Heard et al. 1996; Santiago & Edelman 1985); pulmonary vasoconstriction decreases pulmonary perfusion and negatively alters ventilation perfusion ratios and alveolar gas exchange (Lumb 2005). Other important haemodynamic effects would include the reversal of opioid-induced

systemic vasoconstriction and alterations in blood pressure (Hannon & Bossone 1991; Heard et al. 1990; Heard et al. 1996); both of these effects may decrease tissue perfusion and tissue oxygenation.

Serotonin generally causes pulmonary vasoconstriction (Gyermek 1996), an effect that is attributed predominantly to its binding to 5-HT_{2A} and 5-$HT_{1B/1D}$ receptors in the pulmonary vascular bed (Cortijo et al. 1997; Morcillo & Cortijo 1999). However, there also is evidence that serotonin can mediate vasodilatation directly through the activation of 5-HT_4 and 5-HT_7 receptors located on vascular smooth muscle (Morcillo & Cortijo 1999); in sheep activation of 5-HT_4 receptors causes potent endothelium-independent relaxation of pulmonary veins (Cocks & Arnold 1992), and in rabbits activation of 5-HT_7 receptors causes nitric oxide-independent vasodilatation in pulmonary vessels (Morecroft & MacLean 1998). 5-HT_{1A} receptors have not been identified in pulmonary blood vessels, at least so far in humans (Cortijo et al. 1997), rats and pigs (Cortijo et al. 1997; Ullmer et al. 1995), and their agonists do not have any direct action on these vessels (Ogawa et al. 1995). However, 5-HT_{1A} agonists cause systemic vasodilatation (Dabiré et al. 1990). This vasodilatation is believed to occur via central effects on vasomotor centres which result in a decrease in sympathetic tone (Blessing 2004; Dabiré et al. 1990; Ootsuka & Blessing 2003; Saxena & Villalón 1990; Zwieten 1996). This decrease in sympathetic tone is important as it may reverse the activation of sympathetic neurones brought about as a direct effect of the opioids (Roquebert & Delgoulet 1988) and from opioid-induced hypoxia and hypercapnia (Heard et al. 1990; Heard et al. 1996). This decrease in sympathetic tone results in a decrease in systemic and pulmonary vascular resistance, and may increase pulmonary perfusion

thereby improving ventilation perfusion ratios and gas exchange (Kadowitz & Hyman 1973; Nunn 1993).

The decrease in sympathetic tone caused by 5-HT_{1A} agonists not only decreases vascular resistance but it also affects the heart by causing bradycardia (Dabiré et al. 1990; Saxena & Villalón 1990). Other serotonergic ligands also affect the heart. 5-HT_3 receptor agonists cause bradycardia by activating receptors on postganglionic cardiac sympathetic nerve fibres (Dabiré et al. 1990). 5-HT_2 receptor agonists cause tachycardia and positive ionotropy by inducing the release of catecholamines from adrenomedullary chromaffin cells (Saxena & Villalón 1990). 5-HT_4 receptor agonists also cause tachycardia and positive ionotropy, which results in an increase in blood pressure, by activating receptors in the cardiac atria (Kaumann & Levy 2006; Villalón et al. 1991). These effects of the 5-HT_4 receptor agonists appear to occur only in pigs and humans. In other species, including cats, dogs, rats and rabbits, tachycardia is not induced by the activation of 5-HT_4 receptors but rather by the activation of 5-HT_1 and 5-HT_2 receptors (Langlois & Fischmeister 2003).

Although the specific cardiovascular effects of serotonin and its ligands have been determined, little is known about how these effects alter cardiac output or tissue perfusion. In spite of the many studies on the respiratory neuronal effects of serotonin during opioid-induced respiratory depression, few, if any, have measured the crucial physiological variables of PaO_2 and $PaCO_2$. Additionally, very few of these studies have been done in unanaesthetised animals without the confounding effects of general anaesthetics. Furthermore, most of the studies done to date have been done in laboratory animals and nothing is known about the effects of serotonin and its

ligands in other species including wildlife. Therefore, nothing is known about how the cardiorespiratory effects of serotonin and its ligands influence blood and tissue oxygenation and carbon dioxide removal in animals that receive opioids for clinical applications.

1.3 Consequences of capture-induced hyperthermia and opioid-induced respiratory depression

In all animals there is a lower and upper critical body temperature, also known as the critical thermal minimum or maximum, beyond which cellular damage occurs (Leon 2007; Willmer et al. 2005). In most placental mammals the upper critical limit is between 42˚C and 44˚C (Willmer et al. 2005). However, there is no set upper body temperature that will predict thermal injury in an individual and sometimes this injury occurs at temperatures below a proposed upper critical temperature for that species (Leon 2007). Heat, or heat cytotoxicity, causes cellular injury by causing disruption to metabolic pathways, disturbances in cellular energy supplies, and the degradation and aggregation of proteins which leads to the breakdown of enzymatic processes (Willmer et al. 2005; Yan et al. 2006). It also affects membrane structures so that various transport systems into and between cells are disrupted, thereby upsetting intracellular conditions and cellular metabolism (Willmer et al. 2005; Yan et al. 2006). To complicate matters further, heat causes an increased need for oxygen and energy substrates as it increases cellular metabolism (Willmer et al. 2005; Yan et al. 2006).

The cytotoxic, metabolic and hypoxic effects of heat in the body may cause many cells to undergo lethal damage, which could lead to apoptosis or even cellular necrosis (Yan et al. 2006). The consequences of heat-induced cell damage has been studied extensively in humans (Leon

2007; Yan et al. 2006) and in animal models of heat stroke (Damanhouri & Tayeb 1992). Heat stroke in both man and other animals is associated with abnormally high body temperatures and diffuse tissue injury which can involve gastrointestinal failure, renal failure, hepatic failure, disseminated intravascular coagulation, myocardial failure, skeletal muscle cytolysis, encephalopathy, hypoxemia, metabolic acidosis, hyperkalaemia and cardiogenic shock (Bouchama et al. 2005; Haskins 1995; Leon 2007; Yan et al. 2006). One of the consistent findings in heat stroke is that the gastrointestinal barrier is damaged in the early stages (Bouchama et al. 2005; Bynum et al. 1977; Hall et al. 2001) causing a leakage of endotoxins and the subsequent release of inflammatory cytokines (Leon 2007; Yan et al. 2006), which, in conjunction with the release of other inflammatory mediators from damaged tissues, results in a systemic inflammatory response syndrome (Bouchama & Knochel 2002; Leon 2007; Yan et al. 2006). This systemic inflammatory response syndrome, in combination with the effects of heat cytotoxicity, can result in multiple organ dysfunction and failure (Bouchama & Knochel 2002; Haskins 1995).

Heat stroke in man and other mammals can be caused either by passive exposure to hot environmental conditions, which is known as classical heat stroke, or by strenuous physical exercise, which is know as exertional heat stroke (Bouchama & Knochel 2002; Jessen 2001; Yan et al. 2006). Although classical heat stroke can occur in wild animals, especially if they are compromised by dehydration or disease, it is unlikely to play a major role in capture-induced hyperthermia. On the other hand, exertional heat stroke, which often is associated with rhabdomyolysis, hyperkalaemia, hypocalcaemia, myoglobinaemia and myoglobinurea (Haskins 1995), may play a role because these symptoms are often seen post-capture and are also

associated with capture myopathy (Harthoorn 1976). It is for this reason that it has been proposed that heat stroke and capture-induced hyperthermia are synonymous, and that they play a role in capture myopathy (Antognini et al. 1996; Cheney & Hattingh 1987; Ebedes et al. 1996; Gericke et al. 1978; Meltzer & Kock 2006b). However, this proposal is based on the assumption that capture-induced hyperthermia is caused by physical exercise, an assumption that is not always true as capture-induced hyperthermia occurs even when minimal physical exercise occurs (Bakken et al. 1999). Therefore, although capture-induced hyperthermia and heat stroke share some similarities, the mechanisms that cause the increase in body temperature in these two phenomena appear to be distinct. However, both may play a role in the cause of capture myopathy.

Capture myopathy is a common cause of death or morbidity after capture operations (Ebedes et al. 1996; Harthoorn 1976; Meltzer & Kock 2006b). Although it is primarily associated post-mortally with visible pathology of cardiac and skeletal muscles, lesions are not always seen in muscle, and muscle damage is not always the primary cause of death (Ebedes et al. 1996; Harthoorn 1976; Kock et al. 2006). Harthoorn (1976) described differing degrees of capture myopathy which he attributed to differing levels of stress and exertion an animal is exposed to during capture. The most lethal of these is the hyper-acute form which is associated with per-acute death within an hour of capture (Ebedes et al. 1996) and is attributed to heart failure caused by a profound acidaemia and hyperkalaemia (Harthoorn 1976). In the acute form animals die within 12 hours of capture and death is attributed to heart failure or pulmonary oedema (Harthoorn 1976). The sub-acute form is associated with damage to muscle and other major organs, like the lung, liver and kidneys, with death occurring from these complications within

one to two weeks after capture (Harthoorn 1976). In the chronic form death usually occurs only weeks to months after capture and usually arises when animals are re-captured; it is attributed to either the development of the acute form of the disease, or heart failure which results from chronic myocardial fibrosis (Ebedes et al. 1996; Harthoorn 1976).

The primary causes of capture myopathy are believed to be overexertion during a chase and the stress associated with the capture (Ebedes et al. 1996; Gericke et al. 1978; Harthoorn 1976; Hofmeyr et al. 1973; Kock et al. 2006). The pathophysiology of capture myopathy is very similar to that of exertional rhabdomyolysis in man and horses (Aleman 2008; Criddle 2003; Valentine 2008) and Porcine Stress Syndrome in pigs (Mitchell & Heffron 1982), as patients suffering from these syndromes all present with muscle stiffness, hyperthermia, metabolic acidosis, myoglobinuria, increased serum creatine kinase and potassium concentrations. These changes are believed to be brought about by a combination of a stress-induced increase in serum catecholamines (Ebedes et al. 1996; Gericke et al. 1978; Harthoorn 1976), an increase in anaerobic metabolism due to excessive exertion (Ebedes et al. 1996; Harthoorn 1976), and a derangement in metabolism (Mitchell & Heffron 1982). Anaerobic metabolism in muscle cells results in an increase in lactic acid production and a disturbance of the active transport of electrolytes in and out of cells. These effects result in cellular damage and the leakage of myoglobin, cellular enzymes like creatine kinase, and other constituents like potassium, phosphate, lactic acid and other inorganic acids into the blood stream (Criddle 2003; Guyton & Hall 1997; Harthoorn 1976; Meltzer & Kock 2006b). The resultant metabolic acidosis further compounds the hyperkalaemia as hydrogen ions displace potassium ions from cells (Guyton & Hall 1997). This hyperkalaemia, in combination with severe acidaemia, is believed to be the

cause of heart failure in the hyper-acute and acute forms of capture myopathy (Gericke et al. 1978; Harthoorn 1976). Pulmonary oedema, which also is seen in the acute form, is possibly caused by pulmonary hypertension resulting from acidaemia and an increase in sympathetic and adrenal discharge (Harthoorn & Young 1976), but also may occur as a result of heart failure (Harthoorn 1976). Renal failure, which occurs in the acute form, can occur if muscle damage is severe and is brought about by the combination of renal vasoconstriction and the nephrotoxic effects of myoglobinaemia (Criddle 2003; Harthoorn 1976). The damage seen in other organs of animals with capture myopathy appears to occur mainly because of hypoperfusion, which results in anaerobic metabolism and the build up of toxic metabolites (Harthoorn 1976).

It is apparent that anything that would increase anaerobic metabolism during capture would enhance the severity of capture myopathy. When an animal over-exerts itself its ability to metabolise aerobically is limited by the body's cardiovascular and respiratory capacity to deliver oxygen to cells (Guyton & Hall 1997). During capture this capacity may be diminished by generalized vasoconstriction caused by excessive discharge of the sympathetic nervous system following stress (Kock et al. 2006). Importantly, this vasoconstriction may directly affect oxygen and nutrient supply to muscles as the sympathetic nervous system plays a major role in regulating muscle blood flow during exercise (Rowell 1997; 2004; Thomas & Segal 2004). Hyperthermia also will increase anaerobic metabolism as it increases cellular metabolism; for every 1˚C increase in body temperature metabolism increases by 10-12% (Criddle 2003; Guyton & Hall 1997). At body temperatures above 41-42˚C in mammals the oxygen requirement of cells exceeds the cardiovascular capability for sufficient oxygen delivery leading to hypoxia and cellular damage (Grint et al. 2007; Haskins 1995). Anaerobic metabolism also will be increased

by the insufficient supply of oxygen to the lungs, a scenario that may occur during chemical capture, where opioid drugs may cause respiratory depression, and recumbency may hinder lung expansion and ventilation (Kock et al. 2006).

Opioid-induced respiratory depression is important not only in capture myopathy, but for other reasons it also may influence the survival of an animal during and after chemical immobilization. As discussed in section 1.2.2, opioids cause hypoventilation, ventilation perfusion mismatching, shunting of blood in the lungs, and a decrease in oxygen diffusion across the alveolar membrane. The consequence of these effects is hypoxia and hypercapnia (Nunn 1993). Hypoxia means that less energy is available to the cell, and metabolites like hydrogen and lactate ions build up (Guyton & Hall 1997; Nunn 1993). These ions may escape into the circulation where they can cause a metabolic acidosis, but, in the brain, the blood-brain barrier is relatively impermeable to charged ions, so that these ions build up in neurones and cause an intracellular acidosis (Lumb 2005). The consequences of cellular energy depletion and acidosis are the development of gross abnormalities in ion channel function, which leads to abnormal intracellular and extracellular ionic gradients, leading to cell death (Lumb 2005). Although the organ believed to be the most susceptible to hypoxic damage is the brain, and death due to neuronal depression and damage usually occurs (Lumb 2005), under special circumstances other organs may be more susceptible (Wilson & Benuhof 2005). During anaesthesia, the heart is the organ that is most susceptible to hypoxic damage because hypoxaemia generally promotes cardiac irritability and arrhythmias as the increase in oxygen demand, caused by sympathetic and catecholamine myocardial stimulation, and the decrease in oxygen supply, tips the normal balance between myocardial oxygen supply and demand (Wilson & Benuhof 2005). In addition, if hypoxaemia is severe, with

arterial oxygen haemoglobin saturations below 60%, the cardiovascular system is severely affected and blood pressure falls rapidly due to vasodilatation. Shock eventually develops, and the heart either fibrillates or arrests (Wilson & Benuhof 2005).

Hypercapnia also causes direct depression of both the myocardium and vascular smooth muscle, but at the same time it causes a reflex stimulation of the sympathoadrenal system which usually compensates for any cardiovascular depression (Lumb 2005). However, the reflex sympathoadrenal stimulation further exacerbates myocardial oxygen demand and may cause arrhythmias (Lumb 2005; Wilson & Benuhof 2005). Hypercapnia may further exacerbate hypoxia by diluting oxygen concentrations in the alveoli and shifting the oxygen haemoglobin dissociation curve to the right (Lumb 2005; Wilson & Benuhof 2005). It also can cause generalised pulmonary vasoconstriction which may alter the diffusion of oxygen across the alveoli membrane (Lumb 2005). Finally, if severe, with the arterial partial pressure of carbon dioxide ($PaCO_2$) above 100mmHg, hypercapnia will cause profound neuronal depression which may result in narcosis and death (Guyton & Hall 1997; Lumb 2005; Wilson & Benuhof 2005).

In summary, there is a distinct interplay between the side-effects of capture-induced hyperthermia and opioid-induced respiratory depression. The precise role each of these has to play in the development of capture-related morbidity and mortality has not been defined. Furthermore, little is known about the effects of capture-induced hyperthermia and the role it plays in capture myopathy, and whether its effects are associated with other adverse events like depression and anorexia in boma kept animals. In addition, it is also not known what affects opioid-induced respiratory depression has on wild animals post capture. However, before these questions are answered we need to establish the cause of capture-induced hyperthermia, and

determine how opioid drugs affect respiratory function and pulmonary performance. We also need to determine whether opioid-induced respiratory depression can be reversed adequately without altering the immobilizing and sedative effects of the drugs used to chemically immobilize wildlife.

1.4 Thesis aims

My interest is in pathophysiological events associated with the capture process that may lead to immediate or delayed morbidity and mortality in wildlife. I have chosen to focus on two of these events, capture-induced hyperthermia and opioid-induced respiratory depression, because they are adverse events that consistently occur during capture procedures, and because both can cause morbidity and mortality.

Chapter 1. Sections 1.1 and 1.3 outline our current understanding of the possible causes and effects of capture-induced hyperthermia. Although hyperthermia is a common occurrence during capture, very little is known about its cause. Consequently, interventions to either prevent or treat capture-induced hyperthermia have been instituted based on speculation that capture-induced hyperthermia is related to either the ambient temperature, the activity levels of the animal, the drug's effects, or the effects of stress, during capture. Therefore, I aimed to investigate the mechanisms and patterns of capture-induced hyperthermia.

Section 1.2 and 1.3 outline our current understanding of the possible causes and effects of opioid-induced respiratory depression and also summarise the current understanding of the role serotonin plays in the regulation of respiration and how its actions can be used to reverse opioid-

induced respiratory depression. Reversal of opioid-induced respiratory depression, without reversal of the opioids desired effects, is of paramount importance to both veterinary and human medicine and anaesthesia. However, few agents are available clinically which meet this criteria, and therefore there is a need for new agents. To date, the focus of the research on reversing opioid-induced respiratory depression has been placed on reversing neuronal depression in the respiratory network. The majority of the studies conducted in this field have assessed only the effects of specific receptor ligands on respiratory neuronal activity or on respiratory rate, rhythm and pattern. Currently no study has examined how the serotonergic ligands effects on cardiorespiratory function influence blood gases and whether these ligands can be used to reverse opioid-induced respiratory depression, without reversing chemical immobilization. I aimed therefore to investigate systematically if serotonergic ligands can be used to reverse opioid-induced respiratory depression, by improving blood gases, in chemically immobilized animals. I also aimed to determine how the opioid etorphine, that is used to chemically immobilize wildlife, affects cardiorespiratory function and pulmonary performance.

Chapter 2. Although the continuous measurement of body temperature in springbok (Fuller et al. 2005) and black wildebeest (Jessen et al. 1994) have provided some insights into body temperature changes during capture, little is known about the pattern and mechanisms of capture-induced hyperthermia. I therefore measured body temperature in impala, at 10-minute intervals, with intra-abdominally implanted temperature-sensitive data loggers, throughout various capture procedures. To determine the role of capture drugs in capture-induced hyperthermia, I captured impala with and without immobilizing drugs. I assessed the effect of ambient temperatures and activity levels on capture-induced hyperthermia by measuring both throughout the capture

procedures. To assess the role of stress, I measured serum cortisol levels during capture, and compared body temperature and stress responses of animals in different groups that had been habituated to various periods of boma housing and handling.

Chapter 3. Many capture drugs are believed to alter thermoregulation (Meltzer & Kock 2006a). I therefore investigated whether different capture drug combinations influence the magnitude of the capture-induced hyperthermia in impala. I also assessed whether the immobilizing effects of the different drug combinations, which influence the time from darting to when the animals became recumbent, influenced the magnitude of the capture-induced hyperthermia. Serum cortisol was used to determine whether the magnitude of the capture-induced hyperthermia could be correlated to the stress response induced by the use of the different capture drug combinations. In addition, heart rate, respiratory rate and peripheral haemoglobin oxygen saturation were measured to determine how the different drug combinations affected the cardiorespiratory system and how they were related to the magnitude of the capture-induced hyperthermia.

Chapter 4. Respiratory depression is one of the major side-effects that is caused by the opioid drugs that are used to chemically capture wild animals (Kock et al. 2006; McKenzie 1993). Promising advances at reversing opioid-induced respiratory depression, without affecting the intended use of opioids, have been made using serotonergic ligands (Manzke 2004; Manzke et al. 2003; Sahibzada et al. 2000). Unfortunately, though, very little is known about the effects of these drugs on cardiorespiratory function, and nothing is known about their effects in ungulates. I therefore investigated the cardiorespiratory effects of two experimental serotonergic ligands, which have differing mechanisms of action, in goats. I chose goats as my subjects as they have

been used before as a successful model for the affects of opioid immobilization on cardiorespiratory function in wild ungulates (Buss & Meltzer 2001; Heard et al. 1990; Heard et al. 1996), and they are easy to handle, therefore pre-immobilization data could be collected from them and they could be restrained with ease if the ligands reversed opioid immobilization. I measured blood gases, respiratory rates, heart rates and blood pressures to determine cardiorespiratory function, and times from injection to recumbency and the quality of immobilization, to determine the effects of these serotonergic ligands on chemical immobilization.

Chapter 5. If serotonergic ligands are to be used to reverse opioid-induced respiratory depression during game capture, their effects need to be demonstrated during an actual capture procedure, and the drugs used need to be available commercially. At present, no commercially-available serotonergic drugs are able to reverse opioid-induced respiratory depression in humans (Lötsch et al. 2005; Oertel et al. 2007). I therefore investigated whether three commercially available serotonergic drugs, which have differing mechanisms of action, could be used to reverse opioid-induced respiratory depression in chemically-immobilized impala. To evaluate respiratory function more extensively than previously (chapter four) I measured blood gases, respiratory rates, minute ventilation, tidal volumes, oxygen consumption and carbon dioxide production. To determine the effects of the serotonergic drugs on chemical immobilization I evaluated the quality of the immobilization by measuring the movements of the animals.

Conclusion. In the conclusion I aim to give an overview of the findings that I have made and place these findings in context with existing knowledge. I will also discuss the significance of

these findings and make recommendations as to how they may be used to improve wildlife capture. I will also discuss the future and highlight the relevance of the findings of this thesis.

CHAPTER 2

Hyperthermia in captured impala (*Aepyceros melampus*): A fright not flight response

Meyer LCR, Fick LG, Matthee A, Mitchell D and Fuller A. Journal of Wildlife Diseases 44, 404-416, 2008

Journal of Wildlife Diseases, 44(2), 2008, pp. 404–416
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HYPERTHERMIA IN CAPTURED IMPALA (*AEPYCEROS MELAMPUS*): A FRIGHT NOT FLIGHT RESPONSE

Leith C. R. Meyer,[1,3] Linda Fick,[1] André Matthee,[2] Duncan Mitchell,[1] and Andrea Fuller[1]

[1] Brain Function Research Group, School of Physiology, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa
[2] National Zoological Gardens Lichtenburg Game Breeding Centre, PO Box 716, Lichtenburg 2740, South Africa
[3] Corresponding author (email: leith.meyer@wits.ac.za)

ABSTRACT: To investigate the patterns and mechanisms of capture-induced hyperthermia, we surgically implanted 26 impala (*Aepyceros melampus*) with miniature thermometric data loggers, which measured body temperatures continuously throughout capture procedures. Four groups of impala, which were habituated to varying levels of handling and boma-housing, were captured by net restraint or by chemical immobilization. The study took place between July 1999 and December 2005. Irrespective of whether impala were chemically captured, net-captured, or disturbed by exposure to a stressor, they developed a precipitous increase in body temperature. This increase in body temperature was not related to activity levels; animals that had low activity levels before immobilization had larger increases in body temperature compared to those that had high activity levels but were not immobilized ($t=3.6$, $P=0.001$, $n=5$). Similarly this increase in body temperature was not related to environmental heat load at the time of darting and immobilization ($r=-0.05$, $P=0.85$). Body temperature increase also did not depend on whether the animals were captured using drugs or not. However, we found that those animals that were habituated more to handling and boma-housing had smaller increases in body temperatures ($F=37$, $P<0.001$) and smaller stress responses, indicated by lower plasma cortisol concentrations ($F=5.5$, $P<0.05$), and less fractious behavior, compared to those animals that were habituated less or not at all. Therefore we believe that capture-induced hyperthermia in impala is caused predominantly by stress, which induces a rapid rise in body temperature.

Key words: Body temperature, capture, cortisol, habituation, opioid, stress.

INTRODUCTION

Wild animals typically develop an acute hyperthermia when captured (Hofmeyr et al., 1973; Gericke et al., 1978; Cheney and Hattingh, 1987; Kock et al., 1987a, b; Martucci et al., 1992; Montané et al., 2003, 2007). This hyperthermia is not accompanied by the rigor or other correlates of malignant hyperthermia or Porcine Stress Syndrome (Mitchell and Heffron, 1980, 1982), and currently there is no evidence for any peripheral metabolic derangement. Because it has been proposed that the hyperthermia may play a role in capture myopathy and acute death during capture (Gericke et al., 1978; Cheney and Hattingh, 1987; Antognini et al., 1996; Meltzer and Kock, 2006), measures usually are taken to decrease the incidence and severity of capture-induced hyperthermia. In the absence of a proper understanding of the mechanisms of capture-induced hyperthermia, these measures are based on anecdotal evidence, and on unproven assumptions that hot ambient temperatures and exercise during capture are the main causes of this hyperthermia.

Most records of capture-induced hyperthermia are based on single measurements of rectal temperature. To better understand the causes of capture-induced hyperthermia, it is essential to measure body temperatures continuously before, during, and after capture procedures. Continuous and accurate measurement of body temperature is possible through surgical implantation of miniature temperature-sensitive data loggers into the abdominal cavity. The loggers, which measure and record temperature at specified intervals, have been used successfully to examine thermoregulation in a variety of wild antelope species (Mitchell et al., 2002). Although capture-induced hyperthermia is evident in records of body temperature obtained from abdominal loggers in antelope (Jessen et al.,

1994; Fuller et al., 2005), no study has used the technology available for continuous measurements of body temperature to investigate systematically the cause or severity of capture-induced hyperthermia.

We used temperature-sensitive data loggers to measure continuously body temperature during different capture conditions. We hypothesized that the greater the reaction of an animal to the procedures leading to and including its capture, the greater would be the severity of hyperthermia it develops. We assumed that the reaction of the animal would be influenced by its activity level in attempting to escape capture, by the time between initial intervention and completion of capture, and by the familiarity of the animal with human activities. To vary the activity levels and capture times to test our hypothesis we primarily used chemical immobilization and employed immobilization regimens with increasing sedative efficacy, starting with a low dose of a conventional immobilizing opioid and ending with a higher dose of the same opioid plus a tranquilizing adjuvant. In a few animals we measured the consequences of capture without sedation. As we increased sedative efficacy of the immobilizing agents, we also increased the familiarity of the animals with human interference, by handling the animals and increasing the duration of boma-housing of the animals before our trials started. To investigate whether environmental temperatures play a role in capture-induced hyperthermia, we monitored the microclimate conditions during the trials. We chose impala (*Aepyceros melampus*) as our experimental animals because they are known to be highly excitable and reactive to capture and confinement (Knox and Hattingh, 1992). We found that, in impala at least, the major cause of acute capture-induced hyperthermia is related to stress rather than to the thermal effects of chemical immobilization, environmental heat load, or activity levels during capture.

MATERIALS AND METHODS

The results reported emanate from three studies conducted with the primary aim of investigating hyperthermia in captured impala, and the secondary aim of determining the effects of different capture drugs and catecholamine blockage on body temperature in chemically immobilized impala. The effects of the different capture drugs and catecholamine blockage on body temperature are not reported here, but where we report the effects of chemical immobilization on body temperature we focus on the immobilizing drug etorphine and the tranquilizer azaperone. The procedures were approved by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance numbers 1999/90/05, 2001/78/05, 2004/11/05). All studies took place between July 1999 and December 2005 at the Lichtenburg Game Breeding Centre of the South African National Zoological Gardens (26°07′S, 26°07′E), 220 km west of Johannesburg, South Africa.

Animals and surgery

Twenty-six adult female impala were used. The animals were caught from the wild and transported to bomas (holding pens with high wooden pole walls) no less than 2 wk before they underwent surgery. On the day of surgery they were herded into a game capture vehicle or crate where they received a tranquilizer (zuclopenthixol acetate, 50 mg, Clopixol-Acuphase, Lundbeck, Johannesburg, South Africa; or azaperone, 40 mg, Stresnil, Janssen Pharmaceutica, Johannesburg, South Africa; or haloperidol, 15 mg, Kyron Laboratories, Johannesburg, South Africa), intramuscularly (IM) via a pole syringe. Once tranquil, they were captured individually by hand and anesthetized with halothane (Fluothane, Astra Zeneca, Johannesburg, South Africa) in 100% oxygen via a face mask. Once the animal was anesthetized, a 200×100 mm midline area on the ventral abdomen was shaved and sterilized with a mixture of 5% chlorhexidine gluconate (Hibitane, Astra Zeneca, Johannesburg, South Africa) in 100% ethanol. Within this area, a 50 mm midline incision was made through the skin and *linea alba*, and a miniature temperature-sensitive data logger was placed into the abdominal cavity, where it could float freely. The *linea alba* and skin were sutured closed. The surgical wound was coated with a topical tick repellent (Tickgrease, Cypermethrin 0.025% m/m, Bayer Animal Health Pty, Isando, South Africa) and sprayed with a topical antiseptic spray (Necrospray, Centaur

Labs, Johannesburg, South Africa) to prevent infection, fly worry, and myiasis. Each of the impala received a long-acting penicillin-based antibiotic (4–5 ml IM, Peni LA Phenix, Virbac Animal Health, Johannesburg, South Africa), an analgesic and anti-inflammatory medication containing 140 mg/ml ramiphenazone, 70 mg/ml sodium phenylbutazone, and 0.5 mg/ml dexamethazone (4–5 ml IM, Dexa-Tomanol, Centaur Labs, Johannesburg, South Africa), and a long-acting parasiticide, doramectin (5 mg subcutaneously [SC], Dectomax, Pfizer Laboratories, Johannesburg, South Africa). Animals were marked with different colored plastic ear tags for identification.

At the end of the studies, the impala were recaught, and the data loggers were removed using a similar anesthetic and surgical procedure to that used for implantation. After surgery the impala were released back into a large camp where they ranged free.

Body temperature measurements

Temperature was measured at 10-min intervals in the abdominal cavity of the impala with miniature temperature-sensitive data loggers (StowAway XTI, Onset Computer Corporation, Pocasset, Massachusetts, USA). These data loggers had a measurement range of +34 C to +46 C, to a resolution of 0.04 C. The loggers had a mass of ~40 g, and dimensions of ~50×45×20 mm when covered in an inert wax (Sasol wax EXP986, Johannesburg, South Africa). Before implantation the loggers were calibrated individually, in an insulated water bath, against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany) and were found to have an accuracy of better than 0.05 C.

Darting procedure

Impala were darted with either a Telinject air rifle (VARIO 3R, Telinject, California, USA) and Dan-Inject darts (S300 dart with 1.5×30 mm collared needle, Dan-Inject, Børkop, Denmark) or a Sabi 500 dart gun (SABI Werkswinkel t/a Magnum Arms, Nelspruit, South Africa) and a Pneu-Dart dart (Type P, 3 cc, 13 gauge, 25 mm long wire barbed needle, Pneu-Dart, Williamsport, Pennsylvania, USA). All the darts were positioned in the gluteus muscle group. Once the animal was recumbent it was blindfolded and cotton wool was placed in its ears to reduce external sensory stimuli, as is common practice in professional game capture procedures.

Naïve animals

Six nonhabituated (naïve) impala were used to examine body temperature responses to opioid immobilization and net capture (Table 1). After the impala had been implanted with the data loggers, they were released into a fenced 62-ha camp where they ranged freely. On two occasions, separated by two weeks, the impala were herded into a smaller enclosure (0.25 ha) within the 62-ha camp. After 24 hr, in a random order, the impala were darted with either 0.5 mg etorphine hydrochloride IM (M99, Novartis, Johannesburg, South Africa), or with 10–12 mg fentanyl citrate IM (Kyron Laboratories, Johannesburg, South Africa). Three animals were darted in short succession (<3 min in total). The other three animals in the group, which were not darted, remained in the group and were exposed to the presence of humans in the enclosure and to the darting of their companions. They were darted approximately 1 hr after the first three

TABLE 1. Summary of differences in immobilization regimens, activity levels, and habituation between groups.

Group	Opioid	Dose (mg)	Tranquilizer	Dose (mg)	Activity levels[a]	Habituation	
						Boma-housing (mo)	Handling
Naïve $n=6$	Etorphine	0.5	None		+++	None	None
Four month $n=5$	Etorphine	1.0	Azaperone	40	++	4	4×hand caught +tranquilized[b]
Two month $n=9$	Etorphine	1.5	Azaperone	40	+	2	1×immobilization[c]
Five month $n=6$	Etorphine	1.5	Azaperone	40	+	5	2×immobilization[c]

[a] Activity levels from darting up until recumbency. +++ = high-intensity activity; ++ = medium-intensity activity; + = low-intensity activity.

[b] Each time these animals were handled, they were tranquilized with the long-acting tranquilizer haloperidol.

[c] The animals were immobilized with etorphine to be weighed.

animals had been darted. Once an animal was recumbent a 10 ml venous blood sample was drawn. The animals remained recumbent for 20 min, in a quiet part of the enclosure, after which we reversed the immobilizing effects of the opioids using 1 mg diprenorphine hydrochloride intravenously (IV) (M5050, Novartis) for the animals that received etorphine, and 20 mg nalorphine hydrochloride IV (Kyron Laboratories) for those that received fentanyl. Once fully conscious the first three impala were released back into the enclosure to join their companions, and only after the second three impala were reversed were all the animals released back into the 62-ha camp. The time from darting to the opioid reversal, and release of the animals, was approximately 35 min. On one occasion, two of these impala were chased by a vehicle into capture nets, where they were restrained, blindfolded, and cotton wool was placed in their ears for 15 min before they were released back into the 62-ha camp.

Four-month habituated animals

Five impala were used in a study with a secondary aim of investigating the effects of catecholamine blockade after chemical capture (results reported from control animals only; see below). The impala had been housed in a 30×50 m boma for 4 mo before the experiments. During this 4-mo period, the impala became accustomed and adapted (habituated) to the presence of humans, who provided fodder (lucerne) and cleaned the bomas. On four occasions (Table 1) during the 4-mo period, these animals had been herded into a game capture vehicle, where they were handled, physically restrained, and then injected with the long-acting tranquilizer haloperidol (5–20 mg, according to mass, Kyron Laboratories), and transported to test for the effects of transport on body temperatures (see Wimberger, 2005).

On our experimental days, we herded the group of impala into an adjacent 10×20 m boma, to ensure reliable darting from a closer range. Each impala in the group was darted on two occasions, separated by 2 wk, with 1 mg etorphine and 40 mg azaperone. Once an impala had been immobilized, it was removed from the group and placed in an adjacent boma where it received a bolus IV injection of a catecholamine blocker or a control (2 ml dimethyl sulphoxide, Merck Chemicals, Gauteng, South Africa). After 20 min, we reversed the effects of etorphine using 2 mg diprenorphine hydrochloride and returned the impala to the rest of the group. The time from darting to reversal, and release of the animals, was approximately 30 min. The human activity in the boma, from the time of darting to removal of the immobilized animal, caused agitation and attempted escape in the other impala, even though, at the time, there was no direct interference with them. We did not collect blood samples from these animals because they had received catecholamine blockers, which we expected would alter the normal plasma cortisol changes (Liu et al., 1991).

Two- and 5-mo habituated animals

Fifteen impala were used at different times over a 2-yr period. The animals were housed, in 5×10 m bomas, with a maximum of three animals per boma. They were fed lucerne every second day, and the bomas were cleaned regularly. Nine animals were exempt from our experimental interventions for 2 mo, except for a single occasion on which they were immobilized to be weighed (Table 1). The other six animals were exempt from interventions for 5 mo, except for two occasions on which they were immobilized to be weighed (Table 1). After their exempt period, each animal was darted IM on four occasions, fortnightly in a random order, with four different combinations of opioid plus adjuvant, namely, 1.5 mg etorphine+40 mg azaperone, 1.5 mg etorphine+2 mg medetomidine hydrochloride (Domitor, Novartis, Johannesburg, South Africa) 1.2 mg thiofentanyl oxalate (A3080, Wildlife Pharmaceuticals, Karino, South Africa) +40 mg azaperone, and 1.2 mg thiofentanyl+2 mg medetomidine. We darted three animals shortly after each other, within 3 min of the first dart, with about 20 min between the groups of three. Once each animal was recumbent, we drew a 10 ml venous blood sample. After 30 min we reversed the effects of the drugs, using 3 mg diprenorphine IV for etorphine, 12 mg naltrexone hydrochloride IV (Trexonil, Wildlife Pharmaceuticals) for thiofentanyl, and 10 mg atipamezole hydrochloride IM (Antisedan, Novartis) for medetomidine. There is no recognized reversing agent for azaperone, and 40 mg of azaperone alone did not immobilize or sedate the animals.

Plasma cortisol

Blood samples were drawn from either the cephalic or jugular veins, into a lithium heparin tube (BD Vacutainer Systems, Plymouth, UK). In the field, the samples were kept on ice until they could be centrifuged to separate the plasma. The plasma was stored in a −70 C freezer, until thawed for cortisol concentrations to be determined by radioim-

munoassay (Coat-A-Count Cortisol Kit, Diagnostic Products, Los Angeles, California, USA).

Climatic data

Climatic data at the study site were obtained from a nearby (<1 km) open area with a Hobo portable weather station (Onset Computer Corporation). For the study of the "1–5-mo habituated animals," dry-bulb and black globe temperatures were measured in the bomas, at 2-min intervals, with a Hobo Data Logger (H08-007-02, Onset Computer Corporation) connected to a black globe thermometer. Black globe temperature incorporates air temperature, solar radiation, and wind speed and therefore provides the best single index of environmental heat load.

Data analysis

Results are reported as mean ±SD, and a $P<0.05$ was considered statistically significant. Reported results were derived only from animals that received etorphine or etorphine +azaperone. Changes in body temperature were calculated as the difference between maximum body temperature attained after darting and the mean body temperature over the 30 min before darting. A Pearson's correlation was used to determine the relationship between the body temperature changes and the environmental temperature and between the body temperature changes and times to recumbency across the groups. An unpaired Student's *t*-test was used to test for differences in body temperature changes between animals that were darted and those that were not darted in the "4-mo habituated animals," and body temperature changes in animals that were not darted between the "naïve" and "4-mo habituated group." A one-way analysis of variance (ANOVA) followed by a Student Neuman Keuls (SNK) post-hoc test was used to test for differences between body temperature changes, plasma cortisol concentrations, and times to recumbency between the different groups of animals.

RESULTS

Naïve animals

In the free-ranging impala, body temperature followed a nychthemeral rhythm (Fig. 1, days 1–6), with a mean amplitude of 1.1 C, minimum temperatures in the morning (~06:30 AM) and peak temperatures, of ~39.5 C, in the late afternoon to early evening (~6:00 PM). In response to darting and immobilization, by an opioid alone, the impala became hyperthermic, with body temperatures increasing within minutes and reaching levels as high as 43.5 C (Figs. 1 and 2). Severe hyperthermia was evident not only in the animals that were immobilized (temperature change 3.2±0.6 C) but also in animals in the same group that were not immobilized (temperature change 2.9±0.5 C; Fig. 2). The profile of the body temperature changes was identical, on each day, in all six animals (Fig. 2), despite the animals being darted at different times and with two different opioids. The hyperthermia, related to darting and immobilization, peaked after the reversal of the opioid drugs and then resolved gradually (Fig. 2).

Two free-ranging impala captured after being chased into a capture net had similar hyperthermic responses (Fig. 3, solid arrow) to those of the animals captured by chemical immobilization (Figs. 1 and 2). However, high-intensity exercise, induced by pursuit in a vehicle, in two earlier unsuccessful attempts to capture the impala in the net caused a rise in body temperature (temperature change ~0.7 C; Fig. 3) much smaller than the rise evident when the animals were captured in the net and handled (temperature change ~3 C to ~4 C). The duration of

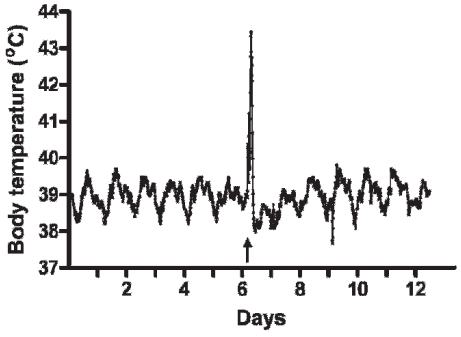


FIGURE 1. Body temperature, measured at 10-min intervals in one, naïve (nonhabituated) female impala over 13 days. The arrow indicates when the impala was immobilized, in this case with etorphine (0.5 mg).

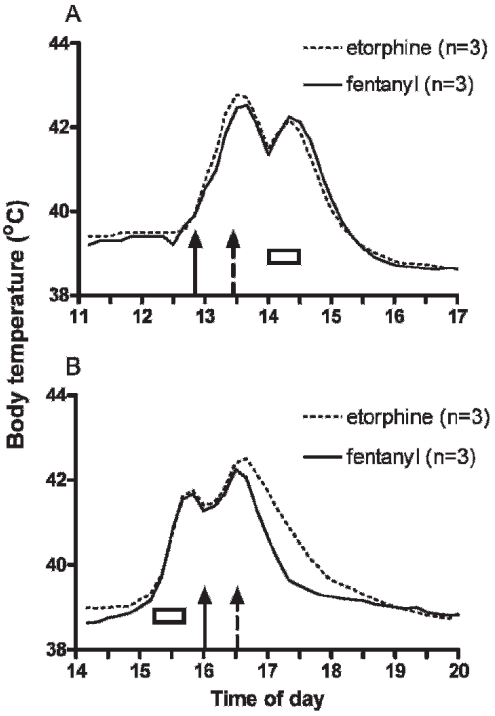


FIGURE 2. Mean body temperatures, measured at 10-min intervals, of six naïve impala before, during, and after immobilization by darting with etorphine (0.5 mg) and fentanyl (10–12 mg), in random order, on two separate nonconsecutive days (A and B). The solid arrows indicate the time at which three impala, in the group of six, were darted and immobilized with etorphine and the dashed arrow the time at which the effects of the etorphine were reversed with diprenorphine in those impala (20 min after darting). The open bars indicate the time at which the other three impala were immobilized with fentanyl. The three impala darted first were in the company of the other three impala in the group, which were to be darted later, until the darted animals became recumbent.

the unsuccessful pursuits (8 and 10 min), and the running speed of the impala, was similar to that of the successful pursuit (10 min).

Four-month habituated animals

Impala that were habituated for 4 mo also developed a hyperthermia when they were immobilized chemically (Fig. 4). Body temperature increases in these impala were significantly smaller than in the "naïve impala" (1.0±0.2 C vs. 3.6±0.6 C; $F=37$, $P<0.001$; Fig. 5A).

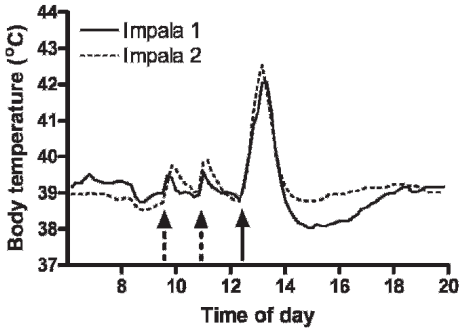


FIGURE 3. Body temperatures, measured at 10-min intervals, of two naïve (nonhabituated) impala. The dashed arrows indicate when both of the animals were chased by a vehicle, which caused full-speed flight and successful escape, followed by a chase, at about the same speed, into a capture net (solid arrow). After the animals became entangled in the net, they were restrained for 15 min by handlers, without chemical immobilization, after which they were released into a 62-ha camp, without further interference.

When an individual in the "4-mo habituated animals" was darted, the other animals in the group, which were actively running, also showed a transient increase

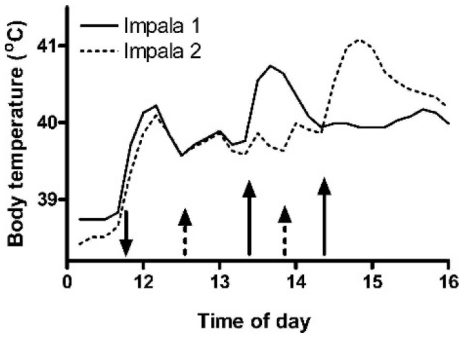


FIGURE 4. Body temperatures, measured at 10-min intervals, of two impala immobilized successively, about 30 min apart, in a group of five animals that were habituated for four months. The downward solid arrow indicates when the group of animals was herded into a novel 10×20 m boma. The following two upward dashed arrows indicate when other impala in the group were immobilized. The upward solid arrows indicate the times at which the two impala were darted (impala 1 depicted by the solid line was darted first). When an impala was immobilized, all the impala stayed together as a group, until the darted animal became recumbent. The immobilized impala was removed from the group to an adjacent boma, while the rest of the impala remained in the original boma where darting took place.

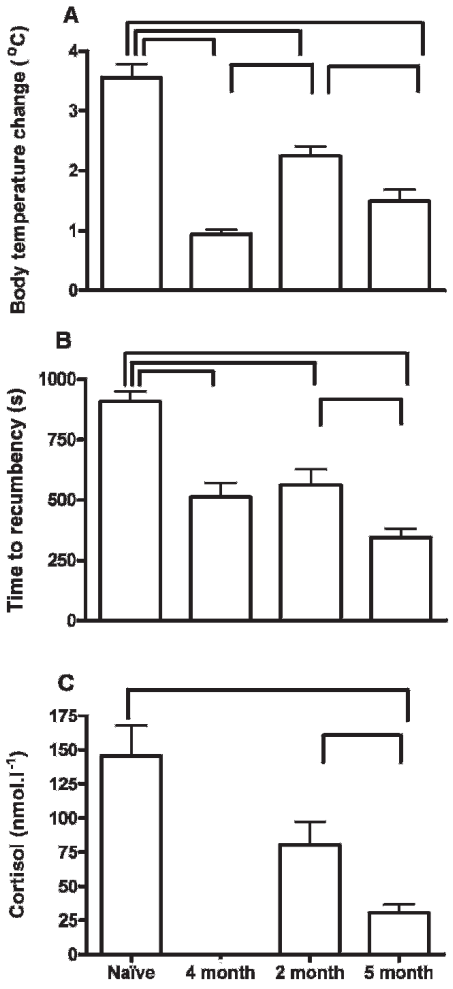


FIGURE 5. Change in body temperature (A), time to recumbency after darting (B), and cortisol concentration (C) in four groups of immobilized impala. "Naïve animals" (n=6) were free-ranging animals that had not been handled or habituated to boma-housing before the darting trials commenced; they were immobilized with etorphine (0.5 mg) only. "Four-month animals" (n=5) had been kept in a large (30×50 m) boma for 4 mo, handled four times, and were immobilized with etorphine (1 mg) plus azaperone (40 mg). "Two-month animals" (n=9) had been kept in small (5×10 m) bomas for 2 mo and had been handled once in that time, and were immobilized with etorphine (1.5 mg) and azaperone (40 mg). "Five-month animals" (n=6) were managed in the same way as the "2-mo animals," except for the longer captivity (5 mo), and were handled twice before been immobilized with etorphine (1.5 mg) plus azaperone (40 mg). Brackets above the bars indicate significant differences between the groups (P<0.05).

in body temperature (an example of this effect is depicted by the dashed arrows in Fig. 4). These increases in body temperature were significantly smaller than the increases in the darted animals, which also ran but were less active than nondarted animals (temperature change 0.4±0.3 C vs. 1.0±0.2 C, t=3.6, P=0.001; n=5; Fig. 4).

Two- and 5-mo habituated animals

When impala that were habituated for 2–5 mo were immobilized with different drug combinations they exhibited an increase in body temperature, with temperature profiles similar to the other immobilized animals described above. When the same amount of etorphine and azaperone were used to immobilize the two groups of impala, the body temperatures in the animals that had been habituated for 5 mo rose significantly less than those that had been habituated for 2 mo (temperature change 1.4±0.4 C vs. 2.1±0.4 C; F=37, P<0.01; Fig. 5A). Even though the doses, per kg body mass, of etorphine and azaperone were similar (F=1.51, P=0.26), the animals that had been habituated for 5 mo became recumbent in a significantly shorter time compared to those that had been habituated for 2 mo (F=11.7, P<0.05; Fig. 5B).

Comparison between groups

The body temperature increases during immobilization were greatest in the "naïve impala" and were generally significantly less, in a descending order, as the impala became more habituated to human handling (Fig. 5A, F=37, P<0.001). Although this trend in body temperature changes between the groups was similar to the trend in the times to recumbency, those animals that were habituated for 4 mo did not become recumbent in a significantly shorter time compared to the "2-mo habituated animals" (F=37, P>0.05; Fig. 5B), and there was no relationship, across the groups, between mean body temperature changes and mean times to

recumbency (r=0.87, P=0.14). The level of activity (see summary in Table 1), from dart placement to recumbency, also was not related to body temperature changes (Fig. 5A).

Plasma cortisol concentrations, after immobilization, were higher in "naïve impala" that received only etorphine, compared to impala that were handled twice, habituated for 5 mo, and received etorphine and azaperone (Fig. 5C, F=5.5, P<0.05). When impala received the same amounts of etorphine and azaperone, those animals that were handled twice and habituated for 5 mo had lower plasma cortisol concentrations compared to those that were handled once and habituated for 2 mo (Fig. 5C, F=5.5, P<0.05).

The body temperature changes that occurred in animals that were not immobilized (these animals were not under the influence of any drugs but with the exception of the actual darting were exposed to the same capture-related stresses) were significantly less in impala that were habituated for 4 mo as compared to "naïve impala" (t=8.05, P<0.0001; Fig. 6).

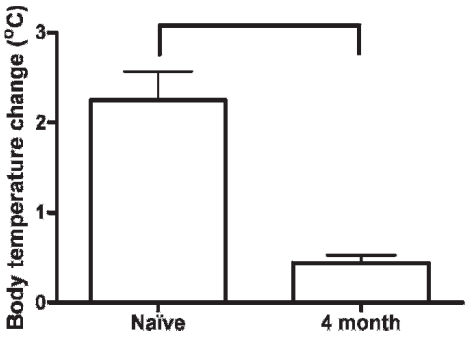


FIGURE 6. Change in body temperature in six "naïve impala" and five "4-mo habituated impala" in response to disturbance when other animals in the group were darted and chemically immobilized. The human activity in the enclosure or boma caused agitation, and attempted escape, in the disturbed animals, even though, at the time, there was no direct interference with the animals. Once the darted animals were immobilized they were either moved to an adjacent boma or were placed in a quiet area of the enclosure, so as to reduce the disturbance of the other members of the group that were not immobilized. Brackets above the bars indicate significant differences between the groups (P<0.05).

Observations

"Naïve impala" appeared to be extremely fractious when approached, and once darted they engaged in high-intensity activity before they became recumbent. Impala that were habituated were less fractious than "naïve impala." Those impala that were habituated for 4 mo, but were darted in the bigger 10×20 m boma, were more active after darting compared to those impala that were habituated for 2–5 mo but darted in the 5×10 m bomas (Table 1). There did not appear to be any differences in activity levels, postdarting, between the impala that had been habituated for 2 mo compared to those that had been habituated for 5 mo, but those impala that had been habituated for the longer time appeared to be less fractious when approached.

Climatic data

Although there were day-to-day and seasonal variations in climatic conditions, and the trials took place throughout the calendar year, the weather conditions did not differ significantly between the trials. Mean dry bulb temperature was 16±8 C and mean black globe temperature was 20±12 C over the study periods. In the "2–5-mo habituated animal study," in which local microclimates were recorded continuously in the boma, the animal's body temperature changes during immobilization were not correlated to dry bulb temperatures (r=−0.19, P=0.5; Fig. 7A) or black globe temperatures (r=−0.05, P=0.85; Fig. 7B).

Morbidity and mortality after immobilization

Three of the "naïve impala" died after experimental trials. All three of these animals had body temperatures that exceeded 43 C during one of the two immobilizations. Macroscopic post-mortem indicated that these animals had lesions indicative of capture myopathy

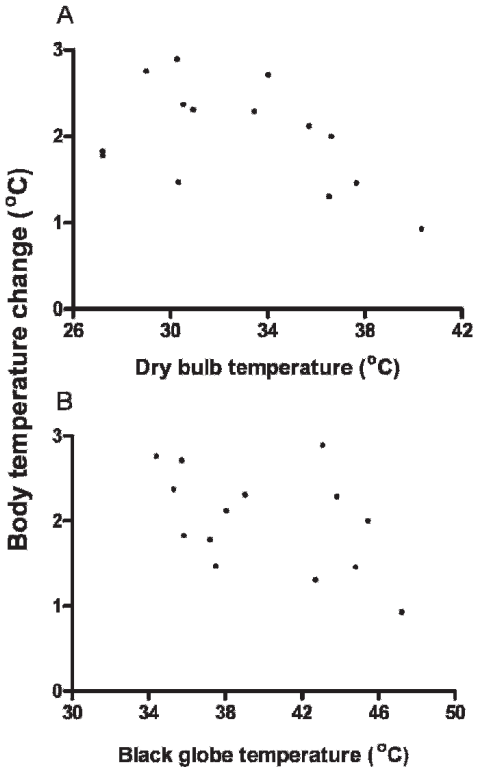


FIGURE 7. Change in body temperature, in response to darting and chemical immobilization, of 15 impala that were habituated for 2 and 5 mo and then darted with etorphine (1.5 mg) and azaperone (40 mg), versus the mean dry bulb temperature (A) and mean black globe temperature (B) measured at 2-min intervals in the bomas during the 30 min of darting and immobilization.

(Young, 1971; Gericke et al., 1978); both their cardiac and skeletal muscles, particularly the semimembranosus and semitendinosus muscles, had extensive areas of dull white necrotic tissue. There also was severe pulmonary and moderate generalized body congestion.

DISCUSSION

By obtaining the first continuous and accurate records of body temperature during a study on capture-induced hyperthermia, we have shown that irrespective of whether impala are chemically captured, net-captured, or disturbed by exposure to a stressor, they developed a precipitous increase in body temperature. This increase in body temperature was well above both the normal 24-hr body temperature patterns (Fig. 1) and the body temperature increase experienced during high-intensity exercise not associated with capture (Fig. 3). The magnitude of the body temperature increase during capture procedures was not related to activity level; animals that had low activity levels during capture had large increases in body temperature compared to those that had high activity levels but were not captured (Figs. 3 and 4). Also, the magnitude of the body temperature rise during capture was not related to environmental heat loads (Fig. 7) and did not depend on whether the animals were captured using drugs or not. Irrespective of whether animals were chemically captured, net-captured, or disturbed by exposure to a stressor, their body temperatures rose in a similar way (compare animals darted to those not darted in Figs. 2 and 3).

The main factor that appeared to influence the magnitude of the body temperature increase was the level of the stress response to capture. Those animals that were habituated more, predominantly by handling procedures and less so by boma-housing, had smaller changes in body temperatures and smaller stress responses, indicated by lower plasma cortisol concentrations and less fractious behavior, compared to those animals that were habituated less or those that were not habituated (Fig. 5). Similarly, those animals that were better habituated were less fractious and had smaller changes in body temperature when they were exposed to capture-related events without themselves being chemically captured (Fig. 6). Therefore capture-induced hyperthermia in impala was not related to the effects of drugs, environmental conditions, or activity, but rather appeared to be strongly related to the level of stress in response to capture.

Hyperthermia is a common sequel when wild animals are captured (Burroughs and McKenzie, 1993). A large

magnitude or prolonged duration of hyperthermia may result in mortality, or may compound capture-related pathologies like capture myopathy (Gericke et al., 1978; Antognini et al., 1996). In our study employing naïve impala, three animals whose temperatures were greater than 43 C died subsequently with macroscopic lesions indicative of capture myopathy. Similarly, two tsessebe (*Damaliscus lunatus*) that had been captured by manual restraint in nets and had body temperatures greater than 43.7 C died acutely without any abnormal macroscopic postmortem findings (Wimberger, 2005). Because of the morbidity and mortality associated with hyperthermia, it is recommended that capture operations take place during only the cooler times of the day (Murray et al., 1981) when air temperatures are below 25 C (Meltzer and Kock, 2006). This recommendation ignores the influence of radiant heat load on animals and does not consider that body temperatures of antelope, following a 24-hr rhythm, are greatest in the late afternoon to early evening when air temperatures are low. Also, there is little evidence that capture-induced hyperthermia is related to environmental heat load, and, to the best of our knowledge, only one study has shown that ambient temperature may influence body temperature during capture (Cheney and Hattingh, 1987). In that study impala were immobilized for 90-min, and ambient heat was most likely to have influenced body temperature due to the prolonged effects of the immobilizing agents, which would have caused the animals to become thermally labile over that time. Our animals were immobilized for a shorter duration (20–30 min), so it was unlikely that ambient heat load would have significantly influenced body temperature.

Although there is evidence that opioid drugs, including etorphine, cause a change in body temperature that is dose-dependent and profoundly influenced by environmental temperature in small animals (Rosow et al., 1980; Clark and Lipton, 1985), it has been suggested that etorphine, when used to immobilize wildlife, causes hyperthermia not through any action on the opioid receptors but through its adrenergic activity (Meltzer and Kock, 2006). The adrenergic activity of etorphine (Roquebert and Delgoulet, 1988) may well have contributed to the hyperthermia that occurred during chemical immobilization, but it cannot account for a similar magnitude of hyperthermia in the animals during net capture, or the hyperthermia that occurred in animals that were not immobilized but disturbed by the presence of humans in a confined area when other members of their group were immobilized. It also has been suggested that there is an increase in muscle activity, and thus an increase in heat production, in animals experiencing the excitement phase of chemical immobilization before they become recumbent (Burroughs and McKenzie, 1993; Meltzer and Kock, 2006). This physical activity again may contribute to the overall hyperthermia, but the contribution to the overall change in body temperature during capture is likely to be small (Bakken et al., 1999).

The major factor contributing to the body temperature elevation appears to be closely associated with the level of the stress response in the animals. Chemical immobilization of wild animals clearly evokes a stress response (Cheney and Hattingh, 1987; Morton et al., 1995; Meltzer and Kock, 2006), and in chemically immobilized impala there is a distinct elevation of stress response variables in plasma (Cheney and Hattingh, 1987) from the "normal" values obtained from brain-shot animals (Hattingh et al., 1988). Our impala had comparable increases in plasma cortisol concentrations compared to captured impala in a previous study (Cheney and Hattingh, 1987). Although there is no objective measure of stress levels in animals, biochemical variables can be used to indicate the level of response to a stressor (Hattingh, 1988;

Hattingh and Petty, 1992). Morton et al. (1995) showed that plasma cortisol concentrations provided a relatively good indication of the levels of the stress response in captured impala and other antelope species. They also found that when antelope were housed in bomas for a protracted time (35 days) they became habituated, and their response to a stressor, indicated by plasma cortisol concentrations, decreased. On the contrary, Knox et al. (1990) found that boma-housed impala that were physically restrained in nets every week for 8 wk did not show a statistically significant decrease in stress hormone response over that time. Plasma cortisol and catecholamine concentrations may have been lower if the duration of their study had been longer and less stressful procedures, such as chemical immobilization, had been used (Hattingh and Petty, 1992). Although we did not measure plasma cortisol concentrations when we initially caught our impala from the wild, and therefore cannot compare the differences in plasma cortisol concentrations in individual animals over time, we believe that habituation occurred in our animals because plasma cortisol concentrations were lower in animals that were handled more, and boma-housed for extended periods of time, compared to "naïve animals." The effects of habituation also were evident in the animals' behavior; when animals were approached they were less fractious if they were habituated more compared to if they were habituated less. However, irrespective of whether the impala were habituated or not, the animals displayed a stress response to being chemically immobilized; all the animals had elevated body temperatures and plasma cortisol concentrations compared to normal values.

The procedure of chemical immobilization probably induced a stress response because of the fear induced by the presence of humans, the fright that occurs with darting, and the anxiety accompanying an inability to escape the "perceived danger" of the capture procedure. Stress-induced hyperthermia is a reaction to a stressor, or is caused by anxiety, and is common to many mammalian species (Bouwknecht et al., 2007) and occurs irrespective of changes in ambient temperatures (Oka et al., 2001) or activity (Moe and Bakken, 1997; Bakken et al., 1999; Montané et al., 2003). The precise mechanisms underlying stress-induced hyperthermia are not known, but the hyperthermia does not arise solely as the result of the metabolic and vascular effects of catecholamine release (Mitchell and Heffron, 1980; Nakamori et al., 1993; Oka et al., 2001). Oka et al. (2001) propose that this hyperthermia results from a centrally regulated rise in body temperature. But whether this hyperthermia indeed is centrally regulated or is consequent of a derangement in metabolism, as are malignant hyperthermia and Porcine Stress Syndrome (Mitchell and Heffron, 1980; Mitchell and Heffron, 1982), remains to be elucidated. What is clear from our data, and from studies carried out in laboratory animals (Olivier et al., 2003; Veening et al., 2004), is that the magnitude of the hyperthermia developed during a stressful event is directly related to the stress response of the animal, and that the magnitude of the stress-induced hyperthermia exceeds that of exercise-induced, and probably all other, hyperthermias.

In summary, we have shown that capture-induced hyperthermia in impala is caused predominantly by stress rather than the effects of physical activity that occur before recumbency, environmental conditions, or the effects of immobilizing drugs. Although we believe that stress is the major cause of capture-induced hyperthermia, we do not suggest that physical activity will not alter body temperature. On the contrary, we propose that excessive physical activity superimposed on stress-induced hyperthermia will compound capture-induced hyperthermia, especially if an animal has peripheral vasoconstriction from increased sympa-

thetic activity. Similarly environmental heat conditions, and the effects of the capture drugs on thermoregulation, would compound capture-induced hyperthermia. To reduce capture-induced hyperthermia, and its associated morbidity and mortality, capture techniques that invoke lower stress responses should be used whenever possible; the period of time that an animal is exposed to a stressor should be kept to a minimum, long high-intensity chases should be avoided, and immobilizing drug cocktails should be chosen so as to restrict times and distances to recumbency. During extreme environmental temperatures, prolonged immobilization and post-capture confinement in hot or cold vehicles should be avoided, but short capture procedures that induce minimal stress can be considered if animals are quickly released into stress-free environments.

ACKNOWLEDGMENTS

We thank the National Zoological Gardens of South Africa for allowing us to access their property and resources, and the staff at the Lichtenburg Game Breeding Centre for their help. We also thank our colleagues from the School of Physiology, University of the Witwatersrand, who helped collect data, in particular, P. Kamerman, A. Faurie, R. Hetem, and B. de Witt. This work was funded by a Faculty Research Committee Grant, University of the Witwatersrand, and by the National Research Foundation, South Africa.

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Received for publication 3 April 2007.

CHAPTER 3

Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilization with 4 different drug combinations

Meyer LCR, Hetem RS, Fick LG, Matthee A, Mitchell D and Fuller A.
Journal of the South African Veterinary Association 79, 121-129, 2008

Article — Artikel

Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations

L C R Meyer[a*], R S Hetem[a], L G Fick[a], A Matthee[b], D Mitchell[a] and A Fuller[a]

ABSTRACT

Thermometric data loggers were surgically implanted in 15 impala (*Aepyceros melampus*) to investigate the consequences of chemical capture. Impala were darted and chemically immobilised for 30 min with each of the following drug combinations: etorphine and azaperone; etorphine and medetomidine; thiafentanil and azaperone, and a thiafentanil medetomidine combination. During immobilisation, pulse oximeter readings, respiratory rhythm, the plane of immobilisation and plasma cortisol concentrations were measured and recorded. The impala developed an extremely high rise in body temperature, which peaked 20–30 min after reversal of the immobilisation. The magnitude of the rise in body temperature was similar for all the drug combinations ($F = 0.8$, $P = 0.5$), but the duration of the hyperthermia was shorter when the thiafentanil and azaperone combination was used ($F = 3.35$, $P < 0.05$). Changes in body temperature were related to the time that it took for an animal to become recumbent after darting ($r^2 = 0.45$, $P = 0.006$) and not to the effect of the drug combination on time to recumbency ($r^2 = 0.29$, $P = 0.46$). The relationship between time to recumbency and body temperature change, and also to plasma cortisol concentration ($r^2 = 0.67$, $P = 0.008$), indicated that physiological consequences of capture were related to the duration of exposure to a stressor, and not to the pharmacology of the capture drugs. Although shorter time to recumbency in individuals resulted in the benefit of smaller stress responses and body temperature changes, those individuals were predisposed to developing hypoxia and possibly induction apnoea. When animals are chemically immobilised, reducing the thermal consequences of capture requires limiting the exposure of the animal to a psychological 'fright stress'.

Key words: body temperature, stress, etorphine, thiafentanil, medetomidine, azaperone, stress-induced hyperthermia, impala (*Aepyceros melampus*).

Meyer L C R, Hetem R S, Fick L G, Matthee A, Mitchell D, Fuller A **Thermal, cardiorespiratory and cortisol responses of impala (*Aepyceros melampus*) to chemical immobilisation with 4 different drug combinations**. *Journal of the South African Veterinary Association* (2008) 79(3): 121–129 (En.). Brain Function Research Group, School of Physiology, University of the Witwatersrand, 7 York Road, Parktown, 2193 South Africa.

INTRODUCTION

When wild ungulates are immobilised chemically, using capture drugs in a dart, they typically develop an extremely high rise in body temperature[2,4,6,11,13,17,22]. This rise in body temperature may result in lethal hyperthermia, but it also increases cellular oxygen consumption[12,16] in animals that may already have a negative oxygen balance from drug-induced hypoxia and a high metabolic demand from intense activity during escape attempts. If cellular energy production cannot keep pace with normal cellular needs, normal cellular function and integrity are disrupted, which ultimately could lead to organ failure[12] and capture myopathy[21]. Understanding why body temperature rises is crucial to survival during chemical capture, but very little actually is known about its cause. Meltzer and Kock[21] suggested that the rise in body temperature during chemical capture may be related partly to the effects of capture drugs on the animal's thermoregulatory processes.

The potential effects of capture drugs on an animal's thermoregulation may vary depending on the pharmacological effects of the drugs, and so the use of different drugs could result in different body temperature responses during capture. The aim therefore was to determine the thermal response of impala to chemical capture using different drug combinations used to immobilise wild ungulates. Cardiorespiratory and plasma cortisol responses to capture were also measured. The drug combinations used consisted of a narcotic opiate drug, used to induce catatonic immobilisation and sedation, and a tranquiliser or sedative, used as an adjuvant to further calm the animals. No studies have investigated the differences between the thermoregulatory effects of etorphine and thiafentanil in ungulates. Thiafentanil induces immobilisation more rapidly and has a shorter duration of action than does etorphine[15,20]. Medetomidine and azaperone are supposed to disrupt central thermoregulatory control and cause thermal lability[8,14]; they affect peripheral blood flow differently, and, hence, heat flow to and from the environment. Azaperone causes vasodilation through its alpha-1(α_1)-antagonistic effects, and therefore increases peripheral blood flow[20,32], whereas medetomidine causes vasoconstriction and a fall in blood pressure and cardiac output, thus decreasing peripheral blood flow[9,20]. Impala (*Aepyceros melampus*) were chosen as experimental animals, because they are known to be highly excitable and reactive to capture[18], and because they are known to develop capture-induced hyperthermia[22].

MATERIALS AND METHODS

The procedures were approved by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance number 2004/11/05). The study took place between December 2003 and December 2005 at the Lichtenburg Game Breeding Centre (26°07'S, 26°07'E) of the National Zoological Gardens, 220 km west of Johannesburg, South Africa.

Animals and surgery

Fifteen adult (mean mass of 37.5 ± 3.5 kg) female impala were caught from the wild and transported to bomas (5 m × 10 m holding pens with solid 3 m-high wooden pole walls) no less than 2 weeks before surgery. On the day of surgery, the impala were herded into a game-transport vehicle where they received a

[a]Brain Function Research Group, School of Physiology, University of the Witwatersrand, 7 York Road, Parktown, 2193 South Africa.

[b]National Zoological Gardens Lichtenburg Game Breeding Centre, PO Box 716, Lichtenburg, 2740 South Africa.

*Author for correspondence.
E-mail: leith.meyer@wits.ac.za

Received: November 2007. Accepted: June 2008.

tranquiliser azaperone 40 mg (Stresnil, Janssen Pharmaceutica, Johannesburg, South Africa) or haloperidol 15 mg, (Kyron Laboratories, Johannesburg, South Africa) intra-muscularly (i.m.) *via* a pole syringe. Once tranquil, impala were captured individually by hand and anaesthetised using halothane (Fluothane, Astra Zeneca, Johannesburg, South Africa) in 100 % oxygen delivered *via* a face mask. A 50 mm incision was made through the midline in the ventral abdominal wall and a miniature temperature-sensitive data logger (described below) was placed, without tethering, into the abdominal cavity. Post-surgery, each impala received a long-acting penicillin-based antibiotic (4–5 mℓ, i.m., Peni LA Phenix, Virbac Animal Health, Johannesburg, South Africa), an analgesic and anti-inflammatory containing 140 mg/mℓ ramiphenazone, 70 mg/mℓ sodium phenylbutazone and 0.5 mg/mℓ dexamethasone (4–5 mℓ, i.m., Dexa-Tomanol, Centaur Labs, Johannesburg, South Africa), and a long-acting parasiticide, doramectin (5 mg, subcutaneously (s.c.), Dectomax, Pfizer Laboratories, Johannesburg, South Africa). The impala were marked with different coloured plastic ear tags for identification. Once the impala recovered from anaesthesia they were returned to the bomas where they were housed for the duration of the study.

At the end of the study, the impala were re-caught, and the data loggers were removed under an anaesthetic and surgical procedure similar to that used for implantation. The animals were then released back into the main reserve.

Body temperature measurements

Core temperature was measured at 10-min intervals in the abdominal cavity of the impala with miniature temperature-sensitive data loggers (StowAway XTI, Onset Computer Corporation, Pocasset, MA, USA). The loggers had a measurement range of +34 °C to +46 °C, a resolution of 0.04 °C and a mass of ~40 g (50 × 45 × 20 mm) when covered in an inert wax (Wax EXP986, SASOL, Johannesburg, South Africa). Before implantation the loggers were calibrated individually, in an insulated water bath, against a high-accuracy thermometer (Quat 100, Heraeus, Hanau, Germany); each logger had a calibrated accuracy of greater than 0.05 °C.

Experimental procedure

Experimental trials began 2–5 months after surgery. Trials were conducted using 3 groups of impala. For the 1st group (n = 4) trials took place between February and April (late southern hemisphere summer to early autumn) 2004, for the 2nd group (n = 6) trials took place in February and March 2005 and for the 3rd group (n = 5) trials took place between June and September (mid-winter to early spring) 2005. Throughout the trials, the impala were housed in 5 × 10 m bomas, with a maximum of 3 impala per boma; these bomas limited the amount of escape activity possible during and after darting. The impala received lucerne and water *ad libitum* and the bomas were cleaned regularly, so the impala were accustomed to occasional human presence. During the trials, each impala was darted by dart gun (Sabi 500, SABI Werkswinkel t/a Magnum Arms, Nelspruit, South Africa; Pneu-Dart dart type P, 3 mℓ volume, 25 mm long, wire-barbed needle, Pneu-Dart, Williamsport, United States of America) between 8:00 and 13:00 and each impala was darted on 4 occasions, fortnightly, in a random order. Each time a different cocktail was administered, namely: 1.5 mg etorphine hydrochloride (M99, Novartis, Johannesburg, South Africa) and 40 mg azaperone; 1.5 mg etorphine hydrochloride and 2 mg medetomidine hydrochloride (Domitor, Novartis, Johannesburg, South Africa); 1.2 mg thiafentanil oxalate (A3080, Wildlife Pharmaceuticals, Karino, South Africa) and 40 mg azaperone; and 1.2 mg thiafentanil oxalate and 2 mg medetomidine hydrochloride. The darts were fired into the gluteus muscles. The doses of the drugs, per unit body mass, were 40.1 ± 3.5μg/kg (mean ± SD) etorphine, 32.1 ± 2.8μg/kg thiafentanil, 1.07 ± 0.09 mg/kg azaperone and 53.4 ± 4.7μg/kg medetomidine. Drug dosages were based on dosages recommended by experienced veterinarians working in the wildlife field[1,3].

All impala in a boma were darted within 3 minutes. Time from dart impact to that at which the impala no longer could stand on its own was designated 'time to recumbency'. Once recumbent, each impala was moved to a non-shaded area in the boma, where it was held in a sternal position, with the head positioned so that the neck was aligned with the spinal column and the head was elevated above the thorax with the nose pointing downwards. This positioning allowed for unobstructed eructation of ruminal gas and maintained open upper airways. The impala were blindfolded and cotton wool was placed in their ears to reduce external sensory stimuli. Respiratory rate was measured by counting the number of breaths in a minute. To measure arterial haemoglobin oxygen saturation and heart rate, a veterinary pulse oximeter (Nonin 9847V with lingual sensor 2000SL, Nonin Medical, North Plymouth, USA) was clipped to the wall of the vulva. According to the manufacturer's specifications, saturation was measured to an accuracy of 3 % and heart rate to an accuracy of 2 beats/min. The level of immobilisation was assessed clinically by observing movement and muscle tone.

Twenty-eight minutes after darting, blood samples were drawn from either a cephalic or a jugular vein, into a lithium heparin tube (BD Vacutainer Systems, Plymouth, UK). The samples were kept on ice until they could be centrifuged to separate the plasma. The plasma was stored at –70 °C until thawed for cortisol concentrations to be measured by radioimmunoassay (Coat-A-Count Cortisol Kit, Diagnostic Products, Los Angeles, USA). Thirty minutes after darting, the action of the immobilising drugs was reversed with 3 mg diprenorphine hydrochloride (M5050, intravenously(i.v.), Novartis, Johannesburg, South Africa) for etorphine, 12 mg naltrexone hydrochloride (Trexonil, i.v., Wildlife Pharmaceuticals, Karino, South Africa) for thiafentanil, and 10 mg atipamezole hydrochloride (Antisedan, i.m., Novartis, Johannesburg, South Africa) for medetomidine.

Climatic data

The microclimate in the bomas throughout darting and immobilisation was assessed by measuring dry-bulb (ambient air) and black globe temperatures 1 m above the ground at 2 min intervals by means of a Hobo data logger (H08-007-02, Onset Computer Corporation, Pocasset, MA, USA). Black globe temperature integrates the effects of ambient air temperature, solar radiation and wind speed, and provides the best single index of dry environmental heat load. Water vapour pressure was not measured, but it typically was very low (~1.8 kPa), at the site.

Data analysis

Results are reported as mean ± SD, and $P < 0.05$ was considered statistically significant. Changes in body temperature were calculated as the difference between maximum body temperature after darting and body temperature immediately before darting. The thermal response index was calculated as the time integral of the elevation of body temperature from body temperature before darting. Pearson product-moment correlations were used to compare relationships between all other relevant pairs of variables. Where appropriate, we correlated the responses of the 15 impala averaged over the 4 different drug combinations (n = 15), or the average response of the 15 impala to each drug combination (n = 4).

Repeated measures 1-way analysis of variance (ANOVA) followed by a Student Neuman Keuls (SNK) *post hoc* test was used to test for differences between the different drug combinations in terms of times to recumbency, body temperature changes, thermal response indices, arterial haemoglobin oxygen saturations, respiratory rates and heart rates. A Kruskal-Wallis test was used to compare cortisol concentrations in response to each of the different drug combinations. A Chi-square test was used to test for an association between drug use and induction apnoea. Because of the length of time that it took for some of the impala to become recumbent with some drug combinations (up to 14 min), and because the immobilising drugs were reversed 30 min after darting, we analysed arterial haemoglobin oxygen saturation, respiratory rate and heart rate only over the 1st 16 min of the immobilisation from the time the animals became recumbent, divided into 0–8 min and 8–16 min. To determine the immediate effects of the drugs on arterial haemoglobin oxygen saturation post-darting, the data in the 1st 4 min of immobilisation (0–4 min) were analysed.

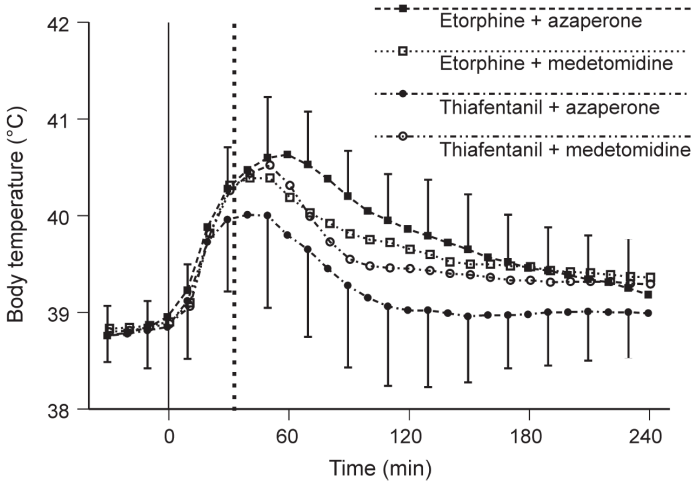


Fig. 1: **Body temperatures** (mean ± SD, $n = 15$), **measured at 10-minute intervals, of impala before, during and after immobilisation with etorphine and azaperone, etorphine and medetomidine, thiafentanil and azaperone, and thiafentanil and medetomidine. The solid line at time 0 indicates the time at which the impala were darted, and the dashed line at 30 min the time at which the immobilising effects of the drugs were reversed, with diprenorphine used for etorphine, naltrexone for thiafentanil, and atipamezole for medetomidine.**

RESULTS

Body temperature and time to recumbency

Body temperature of the impala while they were housed in bomas was 38.93 ± 0.25 °C ($n = 15$), with a fluctuation of about 1.2 °C over 24 h. Body temperature followed a nychthemeral rhythm, with minimum temperatures soon after dawn and peak temperatures in the late afternoon to early evening. Whenever the impala were immobilised body temperature increased rapidly, continued to rise during immobilisation and reached a peak 20–30 min after the effects of the immobilising agents had been reversed (Fig. 1). After this peak, body temperature decreased slowly, but had not stabilised as long as 3 h after reversal of the immobilisation. The magnitude of the body temperature increase was not influenced significantly by the drug combination used to immobilise the impala. There were no significant differences between the mean body temperature changes of the impala following immobilisation with the drug combinations ($F_{3,14} = 0.79$, $P = 0.51$, $n = 15$; Fig. 2A), nor in the thermal response indices calculated over the period of immobilisation ($F_{3,14} = 0.62$, $P = 0.60$, $n = 15$, data not shown). However, the thermal response indices calculated over 2, 3 and 4 h after darting were significantly lower when thiafentanil/azaperone was used as the immobilising agent, than when etorphine/azaperone was used (2 h $F_{3,14} = 3.11$, $P = 0.03$; 3 h $F_{3,14} = 3.62$, $P = 0.02$; 4 h $F_{3,14} = 3.35$, $P = 0.03$; Fig. 2B, all $n = 15$). The reduction in thermal response index associated with immobilisation with thiafentanil and azaperone occurred mainly because body temperatures recovered sooner, rather than because peak temperatures were significantly lower (Fig. 1).

The time for the impala to become recumbent also differed in response to each of the drug combinations ($F_{3,14} = 10.29$, $P < 0.0001$, $n = 15$; Fig. 2C). When impala received thiafentanil they became recumbent more quickly than when they received etorphine, in both combinations (Fig. 2C). There was no significant correlation between time to recumbency and the magnitude of the body temperature changes ($r^2 = 0.29$, $P = 0.46$, $n = 4$; also compare Fig. 2A to 2C), or between time to recumbency and the thermal response indices (30 min $r^2 = 0.49$, $P = 0.30$, to 4 h $r^2 = 0.63$, $P = 0.21$, $n = 4$; also compare Fig. 2B with Fig. 2C), across the different drug combinations. However, there was a positive linear relationship between the mean time to recumbency and mean body temperature change when the responses to the 4 drug combinations were averaged for the individual impala ($r^2 = 0.45$, $P = 0.006$, $n = 15$; Fig. 3A). The mean time to recumbency for the 15 impala was also correlated with the mean thermal response indices over the 30 min ($r^2 = 0.61$, $P = 0.0006$, $n = 15$) and the 4 h period ($r^2 = 0.38$, $P = 0.01$, $n = 15$) after darting.

Because peak body temperatures occurred after the immobilising effects of the drugs had been reversed and because in the field it is impractical to measure body temperatures after the animals had recovered motility, body temperatures at 20 and 30 min after darting were investigated to predict peak body temperature. The body temperature at 30 min after darting ($r^2 = 0.90$, $P < 0.0001$; Fig. 4B) was a better predictor of peak body temperature than was the body temperature at 20 min ($r^2 = 0.87$, $P < 0.0001$; Fig. 4A). Body temperature at 30 min also weakly predicted the 4 h thermal response index ($r = 0.56$, $P = 0.03$), but body temperature at 20 min did not do so ($P = 0.10$). Peak body temperature (y) could be calculated from body temperatures (x) measured during immobilisation using the following equations:

$y = 1.06x - 1.6$ ($Sy.x = 0.33$ °C, body temperature at 20 min),

and

$y = 0.94x + 2.63$ ($Sy.x = 0.19$ °C, body temperature at 30 min).

Because the slope of these equations did not differ significantly from 1 ($F_{1,13} = 0.1$, $P = 0.76$ body temperature at 20 min; $F_{1,13} = 0.41$, $P = 0.53$ body temperature at 30 min) these equations could be simplified by fitting a constrained regression line with a slope equal to 1:

$y = x + 0.79$ ($Sy.x = 0.32$ °C, body temperature at 20 min),

and

$y = x + 0.38$ ($Sy.x = 0.19$ °C, body temperature at 30 min).

Therefore, peak body temperature

could be estimated, by extrapolation, by adding 0.79 °C to body temperature as measured at 20 min, or, better, by adding 0.38 °C to that measured at 30 min.

Although trials were carried out over a range of environmental conditions (dry bulb: minimum = 18.2 °C, mean = 30.1 ± 5.1 °C, maximum = 40.3 °C; black globe: minimum = 24.5 °C, mean = 37.4 ± 5.6 °C, maximum = 47.2 °C), these conditions did not influence the magnitude of the hyperthermia that the impala developed. There was no correlation between the change in the body temperature or the thermal response index and the environmental conditions that prevailed in the bomas at the time of darting and immobilisation, irrespective of which drug combination was used (change in body temperature *vs* dry bulb temperature $r^2 = 0.03$, $P = 0.58$, $n = 15$, Fig. 5A; 4 h thermal response index *vs* dry bulb temperature $r^2 = 0.01$, $P = 0.71$, $n = 15$, Fig. 5B; change in body temperature *vs* black globe temperature $r^2 = 0.02$, $P = 0.65$, $n = 15$, data not shown; 4 h thermal response index *vs* black globe temperature $r^2 = 0.004$, $P = 0.82$, $n = 15$, data not shown).

Plasma cortisol

The mean time to recumbency for the 15 impala was correlated to mean plasma cortisol concentration ($r^2 = 0.29$, $P = 0.008$, $n = 15$; Fig. 3B). The mean plasma cortisol concentration also was correlated to mean body temperature changes ($r^2 = 0.31$, $P = 0.008$, $n = 15$; data not shown) in individual animals. The plasma cortisol concentration, however, did not differ significantly in the impala when they received each of the drug combinations ($P = 0.92$, $n = 4$).

Respiration

There was no significant effect of the different drugs on respiratory rates of the impala over the entire immobilisation period (Fig. 6A). However, induction apnoea (cessation of breathing at the beginning of the immobilisation) occurred in 33 % of the impala that were immobilised with thiafentanil and azaperone, 13 % that were immobilised with thiafentanil and medetomidine, and 7 % that were immobilised with etorphine and medetomidine. It did not occur with etorphine and azaperone. The risk of induction apnoea was significantly different ($\chi^2 = 8.07$, $P = 0.04$) between the 4 combinations of immobilising drugs.

When impala were immobilised with thiafentanil and azaperone, their haemoglobin oxygen saturation was significantly lower in the 1st 8 min of immobilisation than when the other drug combinations were used ($F_{3,14} = 3.74$, $P < 0.05$; Fig. 6B), but it then increased, and became significantly greater, over a period of 8–16 min than when the impala were immobilised with etorphine and azaperone ($F_{3,14} = 2.90$, $P < 0.05$; Fig. 6B). There was no significant difference in the haemoglobin oxygen saturations of the impala given the other drug combinations; the haemoglobin oxygen saturations remained fairly constant throughout the immobilisation period. When the responses of individual impala were analysed, haemoglobin oxygen saturation was lower in impala that had shorter times to recumbency; there was a significant linear correlation between mean haemoglobin oxygen saturation and mean time to recumbency both for the 1st 4 min of immobilisation ($r^2 = 0.49$, $P = 0.004$, $n = 15$; Fig. 3C) and throughout immobilisation ($r^2 = 0.58$, $P < 0.001$, $n = 15$; data not shown). Thus impala that had shorter times to recumbency had smaller increases in body temperature and plasma cortisol concentrations, but lower haemoglobin oxygen saturations, than those with longer times to recumbency (Fig. 3).

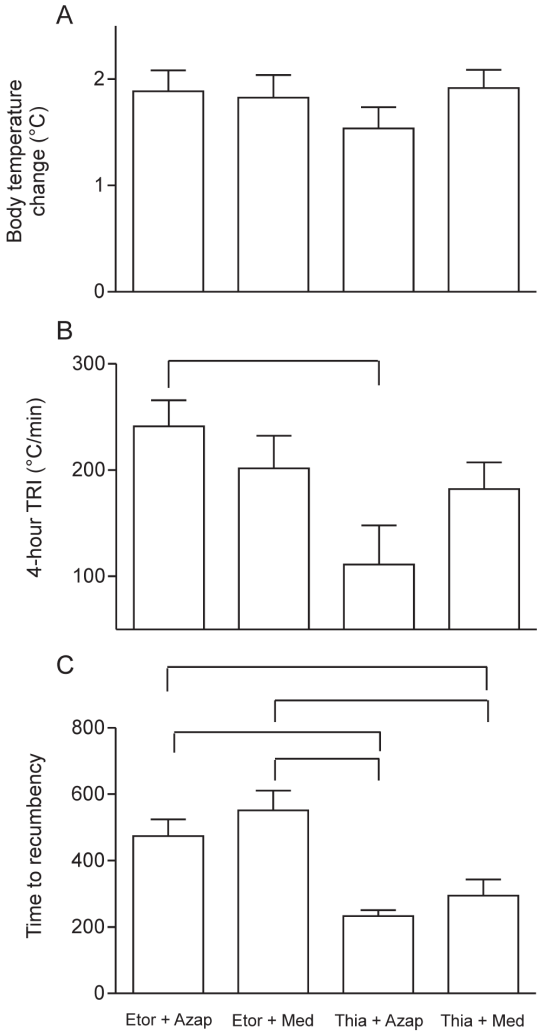


Fig. 2: **Change in body temperature** (A)**, 4-hour thermal response index** (TRI – the time integral of the elevation of body temperature from body temperature before darting) (B)**, and time to recumbency after darting** (C) **in impala** (mean ± SD, n =15) **that were immobilised with etorphine and azaperone** (Etor+Azap)**, etorphine and medetomidine** (Etor+Med)**, thiafentanil and azaperone** (Thia+Azap)**, and thiafentanil and medetomidine** (Thia+Med)**. Bars above the graphs indicate significant differences between the drug combinations** (1-way ANOVA with *post hoc* SNK test, $P < 0.05$)**.**

Heart rate

Irrespective of which drug combination was used to immobilise the impala, their

heart rates were highest at the beginning of the immobilisation and then decreased over the period of the immobilisation (Fig. 6C). When medetomidine was combined with the opiates, the impala had lower heart rates in the 1st 8min of immobilisation than when azaperone was combined with the opiates ($F_{3,14}$ = 8.81, $P < 0.001$; Fig. 6C). For the duration of the immobilisation the impala's heart rates were significantly lower when etorphine and medetomidine, and thiafentanil and medetomidine, were used than with etorphine and azaperone ($F_{3,14}$ = 11.53, $P < 0.05$; Fig. 6C).

Clinical assessment of immobilisation

After becoming recumbent, most of the impala remained immobile, with infrequent and minor movements like chewing (bruxism), ear twitching and tail flicking. However, the impala that received thiafentanil and azaperone moved their legs and bodies from 8 min after becoming recumbent, and some attempted to stand. A few of the impala that received the other drug combinations also tried to move after 8 min but their movements were smaller and attempts to stand were weak.

DISCUSSION

When impala were darted and chemically immobilised for 30 min with 4 combinations of an opiate and a tranquiliser or sedative, they developed a high rise in body temperature that peaked almost an hour after darting (20–30 min after reversal of immobilisation) and gradually declined, in some cases, over more than 3 h after reversal of immobilisation. The rise in body temperature, which could be as much as 3 °C, varied greatly between individual animals and was not correlated to the prevailing environmental heat load, even though air temperature sometimes exceeded 40 °C. The magnitude of the rise also was not determined by the choice of darting drugs. The main factor contributing to the rise in body temperature and body temperature changes over time appeared to be the psychological 'fright stress' that each individual animal experienced during capture, which was directly related to how long the animal was conscious (*i.e.* by the time from darting to recumbency). Irrespective of the drug combination used, some animals became recumbent in a shorter time than did other animals, and those animals had smaller changes in body temperature and plasma cortisol concentrations than did the animals that took longer to become recumbent (Fig. 3A and Fig. 3B). The thiafentanil drug combinations tended to induce recumbency in shorter times than did the etorphine drug combinations (Fig. 2C), but there was a large variability in the times to recumbency between individual animals (Fig. 3). Some impala always became recumbent in about 5 min, regardless of the darting drugs used, while others took twice as long. Because of that variability, there was no statistically smaller mean rise in body temperature in the impala when they received the thiafentanyl combinations (Fig. 2A).

Changes in body temperature therefore appear to have been influenced mainly by an individual animal's stress response to capture, rather than by the pharmacological effects of the immobilising drugs themselves (see Fig. 2A). The shorter the time to recumbency, the less was the hyperthermia, irrespective of how that short time to recumbency was achieved (Fig. 3A). The variable susceptibility of individual impala to the immobilising drugs was demonstrated further by the respiratory depressant effects of the drugs. Those animals that appeared to be more sensitive to the immobilising effects of the drugs, in that they became recumbent in a shorter time and developed smaller rises in body temperature

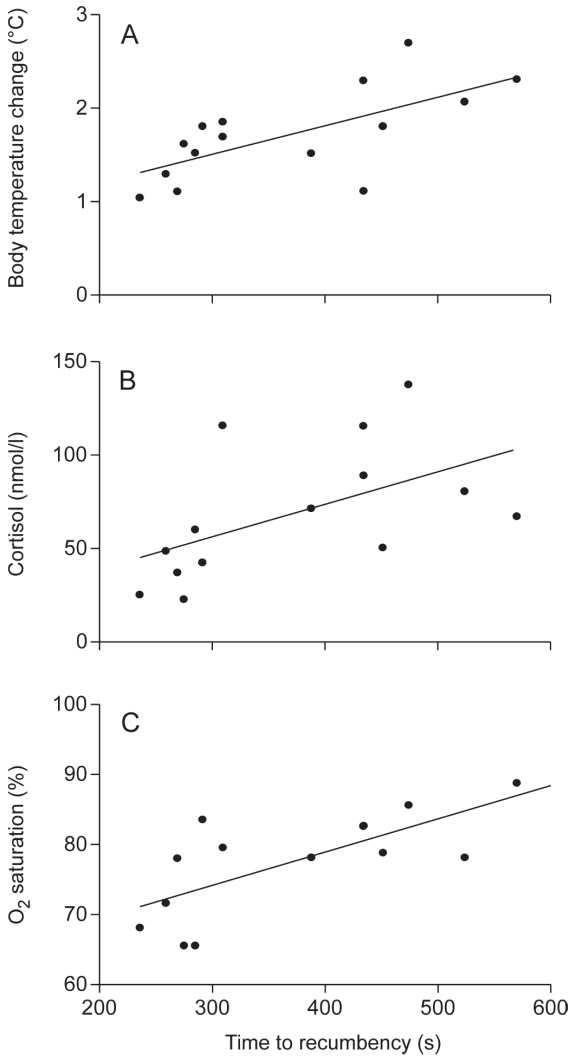


Fig. 3: **Change in body temperature *vs* time to recumbency** (A) (r^2 = 0.45, P = 0.006, n = 15), **plasma cortisol concentration *vs* time to recumbency** (B) (r^2 = 0.29, P = 0.008, n = 15) **and haemoglobin oxygen saturation over the 1st 4 minutes of immobilisation *vs* time to recumbency** (C) (r^2 = 0.49, P = 0.004, n = 15), **of impala in response to darting and chemical immobilisation with etorphine and azaperone, etorphine and medetomidine, thiafentanil and azaperone, and thiafentanil and medetomidine. Responses to the 4 drug combinations were averaged for each impala.**

(Fig. 3A) than those that were less sensitive, also had lower percentage oxygen haemoglobin saturations (Fig. 3C). These animals therefore were more sensitive to the effects of immobilising drugs on the respiratory system. Consequently, a risk of decreasing the time to recumbency is that the animals may develop severe respiratory depression and hypoxia.

Deciding which drug combination causes the least disturbance to the overall physiological function of impala therefore requires balancing the thermal benefits of short time to recumbency with its respiratory risks. Impala that received thiafentanil and azaperone had the smallest duration and extent of hyperthermia. However, the immobilising effects of thiafentanil and azaperone were short-lived and the impala experienced initial severe hypoxia (Fig. 6B), with a high incidence of induction apnoea (33 %). The other drug combinations also caused moderate hypoxia, but this hypoxia was not as severe as that caused by thiafentanil and azaperone. The etorphine drug combinations were less likely to cause induction apnoea than were combinations with thiafentanil, but with these combinations the animals took longer to become immobile (Fig. 2C), and etorphine and azaperone caused prolonged hyperthermia (Figs 1 and 2B). Although thiafentanil and medetomidine did not significantly reduce the magnitude or the duration of the capture-induced hyperthermia below that seen with etorphine drugs combinations, that combination immobilised the impala more quickly and induced only moderate hypoxia (Fig. 6B). Therefore, based on our choice of drugs, doses and capture method, we advocate thiafentanil and medetomidine as the most suitable combination with which to immobilise impala chemically, but caution that the risk of capture-induced hyperthermia, hypoxia and, in some animals, induction apnoea will not be eliminated.

It is possible that different doses of the drugs may have improved the clinical outcome of individual animals, but because the impala reacted to the drugs in a highly variable manner, a drug dose that would improve the clinical outcome in some individuals may well have worsened the outcome in others. It also might have been useful to test the effects of drugs on thermoregulation under controlled conditions, for example in domesticated animals in a laboratory. However, the primary aim of the study was to test the thermal consequences of using different drugs under capture conditions, rather than to systematically investigate the effects of the drugs on thermoregulation.

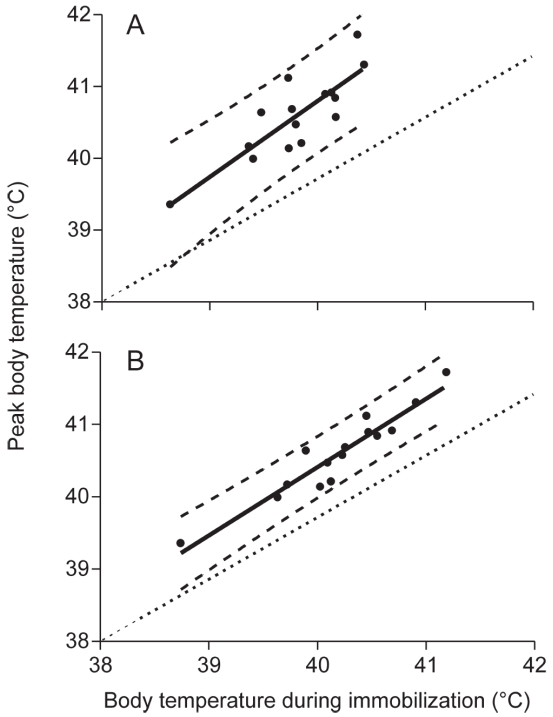


Fig. 4: **Peak body temperature after darting *vs* body temperature at 20 minutes after darting** (A) **and 30 minutes after darting** (B)**, in 15 impala in response to chemical immobilisation with etorphine and azaperone, etorphine and medetomidine, thiafentanil and azaperone, and thiafentanil and medetomidine. Responses to the 4 drug combinations were averaged for each impala. The dotted line is the line of identity** ($y = x$)**, the dashed lines are the 95 % confidence band and the solid lines are the regression lines** (A, $r^2 = 0.90$, $P < 0.0001$; B, $r^2 = 0.87$ $P < 0.0001$).

Opioids and adrenergic ligands have the potential to cause thermal lability and these effects may have become more apparent with longer immobilisation. Although there was no association between environmental conditions and body temperature change, the experimental animals were not exposed to prolonged immobilisation or to severe heat or cold. Impala are known to have a fractious nature and therefore their responses to stress also may be greater than those of other wild ungulate species. However, it is possible that the primary cause of hyperthermia in other wild ungulate species would be the same as that which we found in impala, namely fright stress.

There have been suggestions that capture-induced hyperthermia results partly from the effects of the capture drugs on thermoregulatory processes. Meltzer and Kock[21] proposed that the adrenergic effects of opiate drugs increase metabolic rate and therefore heat production during chemical capture. Although adrenergic effects of opiate drugs have been demonstrated in rats given etorphine[30], these effects have not been linked to body temperature changes. Geller and colleagues[10] showed that etorphine caused a dose-related dual temperature response in rats; low doses caused hyperthermia, medium doses had no effect and high doses caused hypothermia. Rosow and colleagues[31] showed a similar response in mice. They also showed that this response could be altered by changing environmental temperatures[31], implying that the underlying malfunction is thermal lability. The varying effects of opiates on body temperatures are not limited to rats and mice but also occur in other mammalian species, including ungulates[5], and appear to be related to the complex interaction of opiates on opioid receptors both in the central and peripheral nervous system[10,31]. While these effects are not disputed, they are probably not significant during capture with short-term immobilisation.

Like the effects of opiates on body temperatures during chemical capture, the effects of tranquilisers and sedatives on body temperature are not clear. Tranquilisers may affect thermoregulation through several mechanisms including anti-adrenergic effects, inhibition of sweating, and central dopamine antagonism

that disrupts the thermoregulatory set-point causing thermal lability[8]. The tranquiliser we used, azaperone, has potent anti-dopaminergic effects and causes vasodilation through minor α_1-antagonistic effects[20,32]. It would have been expected that both of these effects would have resulted in the impala becoming thermally labile and that their body temperatures, during and after immobilisation, could have been influenced strongly by environmental heat load. Similarly, environmental heat load may have been expected to influence the impalas' body temperatures when medetomidine was used, because central noradrenergic mechanisms are involved in the control of body temperature[7] and α_2-agonists induce thermal lability[14,19]. Even if azaperone and medetomidine did not cause the animals to become thermally labile, some effect on the change in body temperatures would be expected because these drugs have opposite effects on peripheral blood vessel diameters[9,20], and hence blood flows and heat exchange to and from the environment. However, in the impala, the effects of environmental heat load on body temperature were not statistically significant, even over a wide range of environmental conditions. Therefore any effects of environment on body temperature during capture were insignificant, compared with the effects of fright stress.

The psychological processes in fright stress that leads to hyperthermia are not well defined. One factor could be the effects of muscular activity of the animals from darting to immobilisation. To exclude the influence of activity on the body temperatures in this study, the impala were darted in small (5 × 10 m) bomas and they displayed only low-level activity before becoming recumbent. The factor most likely to have induced excessive body temperature rises in the impala was the stress response to capture. Psychological stress is known to induce hyperthermia which causes an acute rise in body temperature similar to that seen in the impala[24,25,28,29]. The precise mechanisms underlying stress-induced hyperthermia are not known, but the hyperthermia does not arise solely as the result of the metabolic and vascular effects of catecholamine release[25,27,28] and rather appears to be centrally regulated in response to a psychological stimulus[28].

It is well known that the behaviour of animals in a group is not uniform and there are distinct differences in stress responses between individual animals when they are placed in captivity[18]. The magnitude of the rise in body temperature that occurred in our impala was related strongly to an individual animal's response to capture. Even though all the animals received similar drug doses per kilogram body mass, some individuals were affected more by the effects of the drugs than were others; some individuals always became recumbent quickly and typically experienced hypoxia. Therefore the variable thermal responses in our animals can most likely be explained by the inter-individual differences in behaviour and stress responses to chemical capture. An animal's susceptibility to the drugs may be related to how 'tame' (not afraid of humans) or how 'wild' (afraid of humans) an animal is. 'Tame' animals generally become recumbent sooner, are more affected by the side-effects of immobilising drugs[20], show a smaller response to stress[26] and have smaller changes in body temperature compared to 'wild' animals[22].

In summary, the acute body temperature elevation that occurs during chemical capture of impala does not appear to be determined primarily by the pharmacological effects of the capture drugs, but rather is related to the animal's psychological stress response to capture. This hyperthermia peaks only after the reversal of the immobilisation and also resolves only hours later. We suggest that limiting the magnitude and duration of this hyperthermia requires minimising the time from first encounter until the animal becomes recumbent. When animals are not confined to a small boma before capture, steps also should be taken to limit additional heat production by decreasing the length, duration and intensity of pre-capture exercise. Restricting capture to cool months of the year, or cool times of the day, will not protect animals from capture-induced hyperthermia; its magnitude is independent of environmental conditions. If animals are severely hyperthermic and especially if they are hyperthermic and hypoxic, steps should be taken to decrease the hyperthermia and ensure that adequate tissue oxygenation takes place. Although our study was not designed to produce the supportive

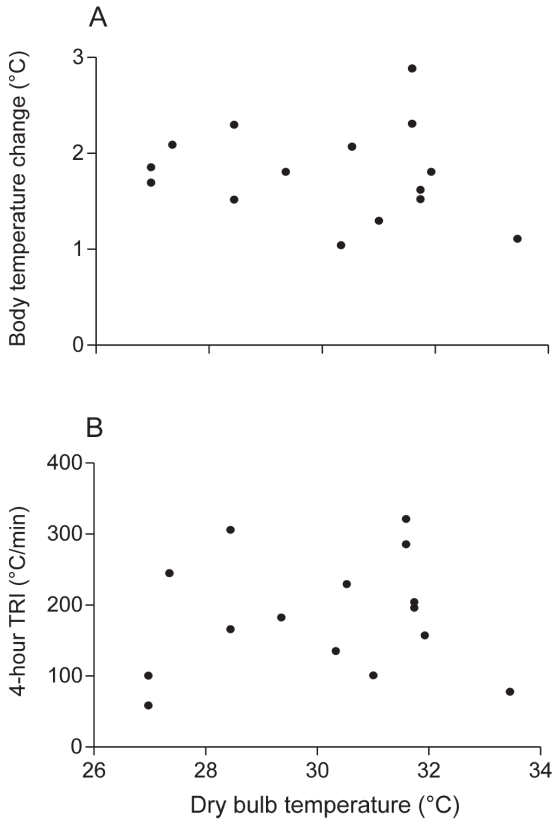


Fig. 5: **Change in body temperature *vs* dry bulb temperature** (A) ($r^2 = 0.03$, $P = 0.58$, $n = 15$) **and 4-hour thermal response index** (TRI – the time integral of the elevation of body temperature from body temperature before darting) ***vs* dry bulb temperature** (B) ($r^2 = 0.01$, $P = 0.71$, $n = 15$) **of impala in response to darting and chemical immobilisation with etorphine and azaperone, etorphine and medetomidine, thiafentanil and azaperone, and thiafentanil and medetomidine. Responses to the 4 drug combinations were averaged for each impala.**

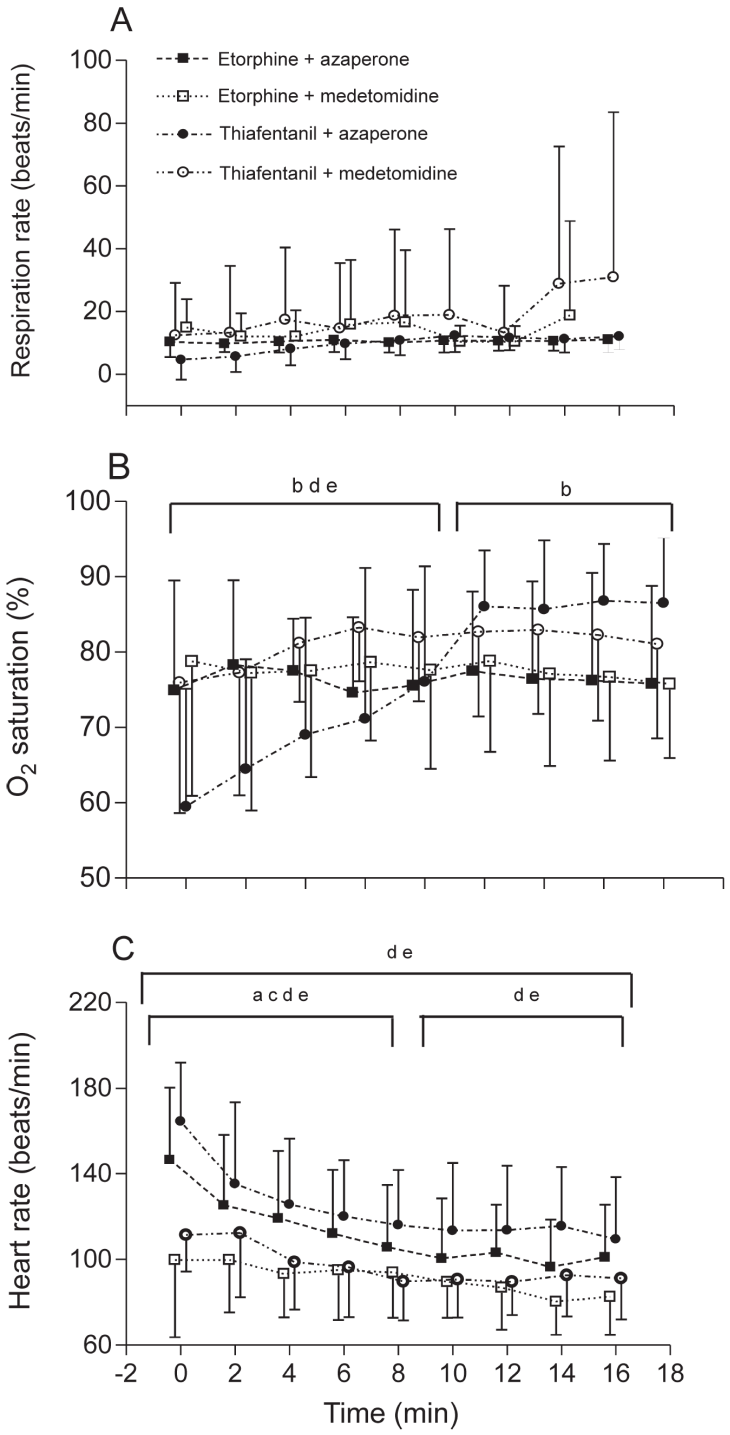


Fig. 6: **Respiratory rate** (A), **haemoglobin oxygen saturation** (B) **and heart rate** (C) **in impala** (mean ± SD, $n = 15$) **from time to recumbency** (time 0). **a, $P < 0.05$ etorphine and azaperone *vs* etorphine and medetomidine; b, $P < 0.05$ etorphine and azaperone *vs* thiafentanil and azaperone; c, $P < 0.05$ etorphine and azaperone *vs* thiafentanil and medetomidine; d, $P < 0.05$ etorphine and medetomidine *vs* thiafentanil and azaperone; e, $P < 0.05$ thiafentanil and medetomidine *vs* thiafentanil and azaperone, 1-way ANOVA with *post hoc* SNK test on means over the periods 0–8 min, 8–16 min and 0–16 min.**

evidence, we believe that, once animals are immobilised, they should be protected from exposure to high or low temperatures. In conclusion, limiting the thermal consequences of capture requires limiting the exposure of the animal to fright stress, and selecting chemical agents that cause rapid recumbency in all individuals without inducing unmanageable respiratory depression and hypoxia.

ACKNOWLEDGEMENTS

We thank the National Zoological Gardens of South Africa for allowing us to access their property and resources, and the staff at the Lichtenburg Game Breeding Centre for their help; our colleagues in the School of Physiology, University of the Witwatersrand, who helped collect data, in particular Brenda de Witt; and Novartis Animal Health for their donation of 1 bottle of etorphine and diprenorphine. This work was funded by a Faculty Research Committee Grant, University of the Witwatersrand, and by the National Research Foundation, South Africa.

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CHAPTER 4

Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats

Meyer LCR, Fuller A and Mitchell D. American Journal of Physiology - Regulatory Integrative and Comparative 290, R405-R413, 2006

Am J Physiol Regul Integr Comp Physiol 290: R405–R413, 2006.
First published September 15, 2005; doi:10.1152/ajpregu.00440.2005.

Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats

Leith C. R. Meyer, Andrea Fuller, and Duncan Mitchell

Brain Function Research Unit, School of Physiology, University of the Witwatersrand, Johannesburg, South Africa

Submitted 22 June 2005; accepted in final form 13 September 2005

Meyer, Leith C. R., Andrea Fuller, and Duncan Mitchell. Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats. *Am J Physiol Regul Integr Comp Physiol* 290: R405–R413, 2006. First published September 15, 2005; doi:10.1152/ajpregu.00440.2005.—Neurophysiological studies have shown that serotonergic ligands that bind to 5-HT_{1A}, 5-HT_7, and 5-HT_4 serotonin receptors in brain stem have beneficial effects on respiratory neurons during opioid-induced respiratory depression. The effect of these ligands on respiratory function and pulmonary performance has not been studied. We therefore examined the effects of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), an agonist of 5-HT_{1A} and 5-HT_7 receptors, and zacopride, an agonist of 5-HT_4 receptors, to establish whether these ligands would reverse opioid-induced respiratory depression and hypoxia without affecting the immobilizing properties of the opioid drug etorphine. When etorphine was used to sedate and immobilize goats, it significantly decreased respiratory rate ($P = 0.013$), percent hemoglobin oxygen saturation ($P < 0.0001$), and arterial oxygen partial pressure [Pa_{O_2}; $F_{(10,70)} = 5.67$, $P < 0.05$] and increased arterial carbon dioxide partial pressure [$F_{(10,70)} = 3.87$, $P < 0.05$] and alveolar-arterial oxygen partial pressure gradient [A-a gradients; $F_{(10,70)} = 8.23$, $P < 0.0001$]. Zacopride and 8-OH-DPAT, coadministered with etorphine, both attenuated the effects of etorphine; respiration rates did not decrease, and percent hemoglobin oxygen saturation and Pa_{O_2} remained elevated. Zacopride decreased the hypercapnia, indicating an improvement in ventilation, whereas 8-OH-DPAT did not affect the hypercapnia and, therefore, did not improve ventilation. The main beneficial effect of 8-OH-DPAT was on the pulmonary circulation; it improved oxygen diffusion, indicated by the normal A-a gradients, presumably by improving ventilation perfusion ratios. Neither zacopride nor 8-OH-DPAT reversed etorphine-induced catatonic immobilization. We conclude that serotonergic drugs that act on 5-HT_{1A}, 5-HT_7, and 5-HT_4 receptors reverse opioid-induced respiratory depression and hypoxia without reversing catatonic immobilization.

serotonin; etorphine; ventilation; alveolar-arterial oxygen partial pressure gradients

OPIOIDS CAUSE RESPIRATORY DEPRESSION, a particular problem when they are used as analgesics (26, 27) and when they are used to immobilize wild herbivores (7, 16, 40). This respiratory depression may cause hypoxic damage to vital organs (31). Opioids affect the respiratory system mainly through their action on μ-opioid receptors on respiratory neurons in the pre-Bötzinger complex (14, 25), a collection of neurons in the brain stem that generate respiratory rhythm (39). The complex depends on neurotransmitters, including serotonin (5-HT), for the modulation of respiratory rhythm (29). Serotonin enhances activity in respiratory neurons through its action on 5-HT_{1A}, 5-HT_4, and 5-HT_7 serotonin receptors (33). The contrasting actions of opioids and serotonin on respiratory neurons allow for the possibility that serotonergic ligands could alleviate the depressive action of opioids on these neurons.

This possibility has been realized in recent neurophysiological investigations. The serotonergic ligands 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), an agonist at 5-HT_{1A} and 5-HT_7 receptors, and buspirone, a partial agonist at the 5-HT_{1A} receptor, reversed morphine-induced depression of respiratory neurons in anesthetized rats (37). BIMU8, an agonist at the 5-HT_4 receptor, reversed fentanyl-induced depression of respiratory neurons, importantly, without reversing analgesia, in anesthetized rats (25). However, although measurements of neuronal activity may reveal the potential of serotonergic ligands to influence respiration, determining whether such ligands actually improve respiratory function requires measurement of pulmonary performance in the whole animal. Also, because serotonin receptors are widely distributed throughout the body (15), even if serotonergic ligands improve pulmonary performance, they may generate adverse effects elsewhere in the body that may negate that benefit. If they are to be used to alleviate opioid-induced respiratory depression, they should not counteract the intentional effects of the opioids.

Mortality and morbidity resulting from respiratory depression are major problems when opioids are used to immobilize animals. We therefore set out to assess whether the serotonergic ligands 8-OH-DPAT and zacopride could be employed to reverse such depression, using the physiologically relevant index of pulmonary function, namely, arterial blood gas status. Because opioids are used therapeutically much more often to immobilize ungulates than to immobilize small animals, we used goats as an experimental animal. As our opioid, we used the pharmacological agent preferred for immobilization of ungulates, namely, the morphine derivative etorphine, a potent agonist of μ-opioid receptors. Concomitantly, we needed to establish whether the serotonergic ligands would influence etorphine-induced catatonia and sedation in the goats. We hypothesized that 8-OH-DPAT, an agonist at 5-HT_{1A} and 5-HT_7 receptors, and zacopride, an agonist at 5-HT_4 receptors and an antagonist at 5-HT_3 receptors, would reverse opioid-induced respiratory depression and hypoxia without reversing the opioid-induced catatonic immobilization and sedation. Although our investigation was targeted to opioid-induced immobilization, its outcomes clearly would have implications for respiratory depression in patients under opioid analgesia.

Address for reprint requests and other correspondence: L. C. R. Meyer, School of Physiology, Univ. of the Witwatersrand, 7 York Road, Parktown 2193, South Africa (e-mail: meyerlcr@physiology.wits.ac.za).

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METHODS

Animals. Eight healthy adult female boer goats (*Capra hircus*), weighing 40 kg (mean, SD 9), were used. They were housed in climatically controlled indoor pens in Johannesburg, at an altitude of 1,753 meters, on a 12:12-h light-dark cycle. They had access to water ad libitum and were fed on hay and sheep concentrate pellets. The procedures were approved by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance 2004/31/5).

Surgery. After veterinary inspection, anesthesia was induced with an intramuscular injection of 2.5 mg/kg ketamine (Anaket; Bayer Animal Health, Johannesburg, South Africa) and 0.04 mg/kg medetomidine (Domitor; Novartis, Johannesburg, South Africa). The goats then were intubated, and anesthesia was maintained with 1–3% halothane (Fluothane; Astra Zeneca Pharmaceuticals, Johannesburg, South Africa) in oxygen. When inhalation anesthesia was stable, 0.2 mg/kg atipamezole hydrochloride (Antisedan; Novartis) was injected intramuscularly to reverse the effects of the medetomidine. The left lateral aspect of the neck was shaved and prepared aseptically for surgery. The left carotid artery was translocated surgically to a subcutaneous tunnel according to the modified transposition technique described by Orsini and Roby (32), to allow for subsequent repetitive arterial catheterization in conscious animals. After surgery, a pressure bandage was placed over the site for 24 h. The animals were given a month to recover before the experimental trials commenced.

Drugs. Etorphine hydrochloride (M99; Novartis) was injected intramuscularly at a dose of 0.06 mg/kg. This dose adequately immobilized and sedated the goats for 30 min. Both 8-OH-DPAT hydrobromide (Tocris, Bristol, UK) and 4-amino-*N*-1-azabicyclo[2.2.2] oct-3-yl-5-chloro-2-methoxybenzamide hydrochloride (Zacopride; Tocris) were used in their racemic form and were injected intravenously at a dose of 0.5 mg/kg. This dose was established in a pilot dose-response study as a midrange dose that increased the respiratory rate in the goats under etorphine immobilization without causing any harmful side effects. Both 8-OH-DPAT (5 mg/ml) and zacopride (10 mg/ml) were dissolved in sterile injectable water (Kyron Laboratories, Johannesburg, South Africa).

Experimental procedures. The experiment consisted of three trials in which each goat received etorphine + water (control), etorphine + zacopride, and etorphine + 8-OH-DPAT, in random order, at weekly intervals. The goats were weighed 2 days before each trial and were starved for 24 h before the trial to reduce the risk of bloating and regurgitation of ingesta. On the day of the trial, the neck (over the translocated artery) and ears were shaved and disinfected. A 22-gauge intravenous catheter (Introcan; B/Braun, Melsungen, Germany) was placed in an auricular vein and connected to a saline drip (Sabax 0.9% NaCl; Adcock Ingram, Johannesburg, South Africa) for subsequent drug injection. Local anesthetic (2 ml of Lignocaine; Bayer Animal Health) was injected subcutaneously around the translocated carotid artery to desensitize the overlying skin. An intra-arterial catheter (14 G, FA-04014; Arrow, Erding, Germany) was inserted through a shallow skin incision, about 4 mm long, into the carotid artery. A three-way stopcock valve (Sabex, Johannesburg, South Africa) was attached to the catheter and secured to the neck with adhesive tape (Leukoplast, Hamburg, Germany).

Once the catheters were in place, the goat was moved into a trolley (0.6 × 1.5 m), where it was restrained by a handler who held the horns. To measure arterial hemoglobin oxygen saturation and heart rate, a veterinary pulse oximeter (Nonin 9847V with 2000T animal transflectance sensor; Nonin Medical, North Plymouth, MN) was placed on the skin at the ventral tail base and secured with adhesive tape. Saturation was measured to an accuracy of 3% and heart rates to an accuracy of 2 beats/min. A pressure transducer (1210 ICSensor; MSI Sensors, Fairfield, NJ) was connected to one arm of the three-way stopcock valve with 1.19-mm tubing (Portex, Kent, UK), and the transducer was attached to a processor constructed for us (School of Electrical Engineering, University of the Witwatersrand) to measure and log mean arterial pressure every 15 s to an accuracy of 2 mmHg. Rectal temperatures were measured with a thermocouple thermometer (BAT-12; Physitemp Instruments, Clifton, NJ) to an accuracy of 0.2°C and were used to calculate water vapor pressure in alveolar air. A digital stopwatch was used to record times to recumbency and respiratory rates. Recumbency was determined when a goat could no longer stand in a supine position on its own.

The etorphine injection induced immobilization and recumbency. The level of immobilization was assessed clinically by a veterinarian observing movement, neck tone, and vocalization. The goats were held in sternal recumbency by a handler holding the horns so that the neck was aligned with the spinal column and the head was elevated above the thorax with the nose pointing downward. This positioning allowed for unobstructed eructation of ruminal gas and open upper airways. After 30 min, the action of etorphine was reversed by intravenous injection of 0.096 mg/kg diprenorphine hydrochloride (M5050; Novartis). Data recordings started 6 min before etorphine injection (injection time = 0 min) and continued for 40 min after injection. Heart rate, hemoglobin oxygen saturation, rectal temperature, and respiration rate were recorded every 2 min. Respiration rates were measured by counting breaths, visible by movement of the chest and abdominal wall, over a minute.

A 0.5-ml carotid arterial blood sample was drawn 2 min before etorphine injection, at 6, 10, 20, and 30 min after etorphine injection, and 10 min after etorphine reversal. After each sample was drawn, the intra-arterial catheter was flushed with 5 IU/ml heparinized (Heparin; Intramed, Johannesburg, South Africa) saline. Directly after the sample was drawn, a blood gas analyzer (Roche OPTI CCA analyzer + OPTI cassette B; Kat Medical, Johannesburg, South Africa) was used to measure the arterial partial pressure of oxygen (Pa_{O_2}) and carbon dioxide (Pa_{CO_2}) in the sample to an accuracy of 1.3 mmHg for Pa_{O_2} and 0.4 mmHg for Pa_{CO_2}. At the end of each trial, the catheters were removed, and a pressure bandage was placed over the carotid artery for 6 h to prevent hematoma formation in the neck. Once the etorphine trials were completed, the goats were given intravenous injections of 0.5 mg/kg 8-OH-DPAT and 0.5 mg/kg zacopride separately and without etorphine, to assess whether the serotonergic ligands alone had effects on the goats. At the end of the experiment, all of the goats were returned to stock.

All measurements were made indoors, between 0800 and 1300, at an ambient dry bulb temperature between 20 and 22°C and relative humidity between 21 and 24%. Barometric pressures were measured to an accuracy of 0.1 mmHg by using the on-board barometer of the blood gas analyzer, which we had calibrated against a Fortin mercury barometer (Russel Scientific Instruments, Dereham, UK). Barometric pressure ranged from 628 to 634 mmHg.

Data analysis. We used GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA) and Statistica 99 edition (StatSoft, Tulsa, OK) for statistical analyses. All results were reported as means, SD, and $P < 0.05$ was considered statistically significant. The areas between the response curves (over time) to etorphine + water, etorphine + zacopride, and etorphine + 8-OH-DPAT were calculated for respiration rate, heart rate, hemoglobin oxygen saturation, and mean arterial pressure for the first 6-min interval (preetorphine + water/ligand administration), for the first, second, and third 10-min intervals and the entire 30 min after etorphine + water/ligand administration, and for the 10 min after diprenorphine administration. A one-way ANOVA followed by a Student-Newman-Keuls (SNK) post hoc test was used to test for differences between these areas and also for differences in the times to recumbency. A Student's paired *t*-test was used to determine differences within the trials, between pre- and postetorphine + water/ligand administration, and between preetorphine + water/ligand and postdiprenorphine administration. Bonferroni corrections were applied where necessary.

For Pa_{O_2}, Pa_{CO_2}, and alveolar-arterial oxygen partial pressure gradients (A-a gradients), a two-way ANOVA followed by a SNK post hoc test was used to test for differences between responses to pairs of

injections and for differences between pre- and post-etorphine + water/ligand responses and pre-etorphine + water/ligand and post-diprenorphine administration in each trial. The A-a gradients were calculated for an open system (constant pressure) from the formula $F_{I_{O_2}}(P_b - P_{H_2O}) - Pa_{CO_2} - Pa_{O_2}$, where $F_{I_{O_2}}$ is the fractional inspired oxygen (0.209), P_b is the measured barometric pressure (mmHg), and P_{H_2O} is the water vapor pressure of saturated air in the alveoli. P_{H_2O} (mmHg) was calculated as $4.58 \exp [(17.27T_b)/(237.3 + T_b)]$ (3), where T_b is the body temperature taken as per rectum. We assumed that the partial pressure of carbon dioxide in the alveoli was equal to the Pa_{CO_2} (13, 31, 35).

RESULTS

Immobilization. Administration of etorphine caused immobilization and recumbency in all the goats in all three trials. When etorphine was injected with water, it took 93 (SD 13) s ($n = 8$) for the goats to become recumbent. Throughout the 30 min of immobilization, the etorphine administration caused sedation, muscle relaxation with only slight body movements, and occasional vocalization. When 8-OH-DPAT was injected with etorphine, time to recumbency was reduced significantly ($F = 1.4$, $P < 0.05$) to 51 (SD 21) s, but the subsequent degree of immobilization was not qualitatively different from that following etorphine administration with water. Zacopride administered with the etorphine also significantly ($F = 1.4$, $P < 0.05$) reduced the time to recumbency, to 63 (SD 23) s, but zacopride coadministered did alter the immobilizing effects of etorphine: the goats had increased muscle tone, moved more, and vocalized more than when they received etorphine + water. Although the sedative effects of etorphine seemed to have been reduced by zacopride, the animals were unable to stand or engage in any coordinated movement at any time during the immobilization period. Neither zacopride nor 8-OH-DPAT immobilized or sedated the goats when the agents were injected at the same dose but without etorphine. When the ligands were injected without etorphine, the goats became restless, and we were unable to accurately assess any cardiorespiratory variables.

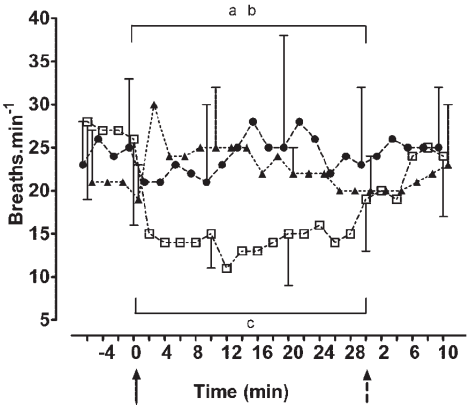


Fig. 1. Drug effects on respiratory rate over time. Values are respiratory rate (means, SD, $n = 8$) of goats injected (solid arrow, time = 0 min) with (intramuscular + intravenous) etorphine + water (□), etorphine + zacopride (●), and etorphine + 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (▲). Dashed arrow (time = 30 min) indicates intravenous injection of diprenorphine. [a]$P < 0.05$, etorphine + zacopride vs. etorphine + water; and [b]$P < 0.05$, etorphine + 8-OH-DPAT vs. etorphine + water [1-way ANOVA with post hoc Student-Newman-Keuls (SNK) test on areas between the curves]. [c]$P < 0.025$, etorphine + water preinjection vs. postinjection (Student's paired *t*-test). Respiratory rates were not significantly different among the trials before the agents were injected ($F = 3.1$, $P = 0.19$).

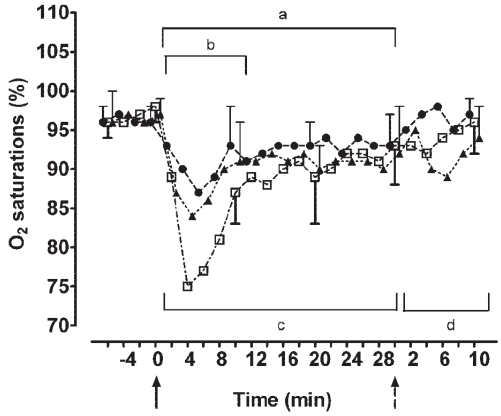


Fig. 2. Drug effects on percent hemoglobin saturation of arterial blood by oxygen. Values are percent saturation (means, SD, $n = 8$) of goats injected (solid arrow, time = 0 min) with (intramuscular + intravenous) etorphine + water (□), etorphine + zacopride (●), and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30 min) indicates intravenous injection of diprenorphine. [a]$P < 0.0125$, etorphine + zacopride vs. etorphine + water; and [b]$P < 0.0125$, etorphine + 8-OH-DPAT vs. etorphine + water (1-way ANOVA with post hoc SNK test on areas between the curves). [c]$P < 0.025$, etorphine + water preinjection vs. postinjection; and [d]$P < 0.025$, etorphine + 8-OH-DPAT preinjection vs. postreversal (Student's paired *t*-test). Saturation values were not significantly different among the trials before the agents were injected ($F = 0.1$, $P = 0.9$).

Respiratory rate. Etorphine administration caused a significant (Student's paired *t*-test, $P = 0.013$) decrease in respiratory rate: before etorphine + water were injected, the respiratory rate was 27 (SD 9) breaths/min ($n = 8$), and after etorphine + water injection, the respiratory rate decreased to 14 (SD 4) breaths/min, averaged over the 30-min immobilization period (Fig. 1). The respiratory rate returned to preinjection rates once the etorphine action was reversed with diprenorphine (Student's paired *t*-test, $P = 0.1$). Zacopride (Student's paired *t*-test, $P = 0.91$) and 8-OH-DPAT (Student's paired *t*-test, $P = 0.4$), coadministered separately with etorphine, both abolished the decrease in the respiratory rate caused by the etorphine administration. Both drugs significantly ($F = 5.65$, $P < 0.05$) increased the respiratory rate over the full 30-min period of immobilization compared with the etorphine + water trial.

Percent hemoglobin oxygen saturation. Etorphine administration resulted in a significant (Student's paired *t*-test, $P < 0.0001$) decrease in the saturation of arterial hemoglobin with oxygen over the 30 min of immobilization (Fig. 2). The decrease in saturation was greatest in the first 10 min of the immobilization. Saturation before etorphine administration was 96 (SD 3)% ($n = 8$) and dropped to as low as 75 (SD 7)% ($n = 8$) after 4 min, with a gradual increase thereafter over time. After diprenorphine injection, saturation returned to near preinjection values (Student's paired *t*-test, $P = 0.5$). Although saturations significantly decreased after the administration of

etorphine + zacopride (Student's paired *t*-test, $P = 0.0025$) and etorphine + 8-OH-DPAT (Student's paired *t*-test, $P = 0.0002$), both zacopride and 8-OH-DPAT attenuated the etorphine-induced decrease in saturation. Over the entire immobilization period, saturation in the goats that received etorphine + zacopride was significantly ($F = 7.18$, $P < 0.05$) higher than that when they received etorphine + water. Saturation in the goats that received etorphine + 8-OH-DPAT was significantly ($F = 10.76$, $P = 0.0015$) higher than that when they received etorphine + water only over the first 10-min interval after administration. Zacopride (Student's paired *t*-test, $P = 0.75$) did not alter the return of saturation to preinjection levels after diprenorphine administration, whereas saturation of the goats that received 8-OH-DPAT + etorphine remained moderately depressed (Student's paired *t*-test, $P = 0.02$).

Partial pressure of oxygen. Figure 3 shows the effect of administration of etorphine, with and without the serotonergic ligands, on Pa_{O_2}. Pa_{O_2} was 69 (SD 4) mmHg ($n = 8$) before etorphine administration. After the injection of etorphine + water, Pa_{O_2} dropped to below 50 mmHg after 6 min. The drop following etorphine + water was significant [$F_{(10,70)} = 5.67$, $P < 0.05$] over the first 20 min of immobilization. Thereafter, Pa_{O_2} gradually increased, and returned to preinjection values [$F_{(10,70)} = 5.66$, $P = 0.5$] after diprenorphine injection. Zacopride and 8-OH-DPAT attenuated, but did not fully abolish, the etorphine-induced decrease in Pa_{O_2}, and even though the Pa_{O_2} values decreased when zacopride and 8-OH-DPAT were injected with etorphine, both drugs maintained significantly [$F_{(10,70)} = 5.67$, $P < 0.05$] higher levels of Pa_{O_2} in the goats in the first 10 min of immobilization. Neither zacopride [$F_{(10,70)} = 5.67$, $P = 0.64$] nor 8-OH-DPAT [$F_{(10,70)} = 5.67$, $P = 0.95$] affected the return of Pa_{O_2} values to preinjection values after diprenorphine administration.

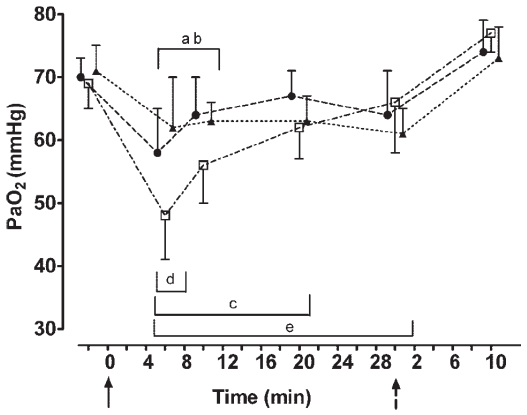


Fig. 3. Drug effects on arterial partial pressure of oxygen (Pa_{O_2}). Values are Pa_{O_2} (means, SD, $n = 8$) of goats injected (solid arrow, time = 0 min) with (intramuscular + intravenous) etorphine + water (□), etorphine + zacopride (●), and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30 min) indicates intravenous injection of diprenorphine. [a]$P < 0.05$, etorphine + zacopride vs. etorphine + water; [b]$P < 0.05$, etorphine + 8-OH-DPAT vs. etorphine + water; [c]$P < 0.05$, etorphine + water preinjection vs. postinjection; [d]$P < 0.05$, etorphine + zacopride preinjection vs. postinjection; and [e]$P < 0.05$, etorphine + 8-OH-DPAT preinjection vs. postinjection (2-way ANOVA with post hoc SNK test). Pa_{O_2} values were not significantly different among the trials before the agents were injected [$F_{(10,70)} = 5.67$, $P > 0.05$].

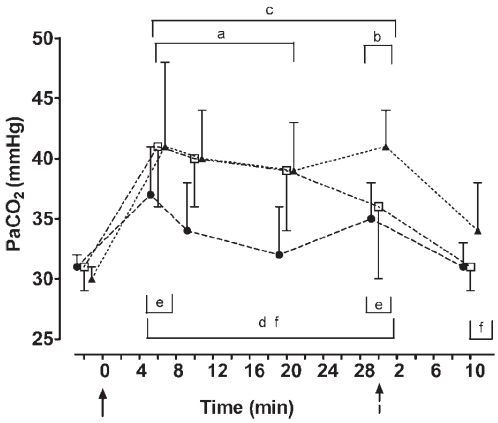


Fig. 4. Drug effects on arterial partial pressure of carbon dioxide (Pa_{CO_2}). Values are Pa_{CO_2} (means, SD, $n = 8$) of goats injected (solid arrow, time = 0 min) with (intramuscular + intravenous) etorphine + water (□), etorphine + zacopride (●), and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30 min) indicates intravenous injection of diprenorphine. [a]$P < 0.05$, etorphine + zacopride vs. etorphine + water; [b]$P < 0.05$, etorphine + 8-OH-DPAT vs. etorphine + water; [c]$P < 0.05$, etorphine + zacopride vs. etorphine + 8-OH-DPAT; [d]$P < 0.05$, etorphine + water preinjection vs. postinjection; [e]$P < 0.05$, etorphine + zacopride preinjection vs. postinjection; and [f]$P < 0.05$, etorphine + 8-OH-DPAT preinjection vs. postinjection/reversal (2-way ANOVA with post hoc SNK test). Pa_{CO_2} values were not significantly different among the trials before the agents were injected [$F_{(10,70)} = 3.87$, $P > 0.05$].

Partial pressure of carbon dioxide. Administration of etorphine resulted in a significant [$F_{(10,70)} = 3.87$, $P < 0.05$] increase in Pa_{CO_2} throughout the immobilization period (Fig. 4). Pa_{CO_2} was 31 (SD 2) mmHg ($n = 8$) before etorphine administration. The highest Pa_{CO_2} value (41 (SD 5) mmHg) occurred 6 min after the etorphine + water injection and gradually decreased over time, returning to preinjection values after diprenorphine injection [$F_{(10,70)} = 3.87$, $P = 0.94$]. Coadministration of 8-OH-DPAT with etorphine had no beneficial effect, and the Pa_{CO_2} levels remained significantly [$F_{(10,70)} = 3.87$, $P < 0.001$] elevated throughout the immobilization. Zacopride coadministration significantly attenuated the rise in Pa_{CO_2} caused by etorphine. The Pa_{CO_2} value for etorphine + zacopride was significantly [$F_{(10,70)} = 3.87$, $P < 0.05$] lower than those for etorphine + water and etorphine + 8-OH-DPAT in the first 20 min of the immobilization period. Zacopride [$F_{(10,70)} = 3.87$, $P = 0.93$] did not alter the return of Pa_{CO_2} values to preinjection values after diprenorphine administration, whereas in the etorphine + 8-OH-DPAT trial, Pa_{CO_2} values did not return to preinjection values and remained moderately elevated [$F_{(10,70)} = 3.87$, $P < 0.05$].

A-a gradient. Figure 5 shows the effect of etorphine administration, with and without coadministration of the serotonergic ligands, on a derived variable, namely, the A-a gradient in the partial pressures of oxygen. The gradient was 21 (SD 3) mmHg ($n = 8$) before administration of etorphine. When etorphine + water were injected, there was a significant [$F_{(10,70)} = 8.23$, $P < 0.0001$] increase in the A-a gradient, which resolved progressively during the time course of the immobilization. Coadministration of 8-OH-DPAT with etorphine abolished the increase in the gradient [$F_{(10,70)} = 8.23$, $P = 0.5$], and indeed,

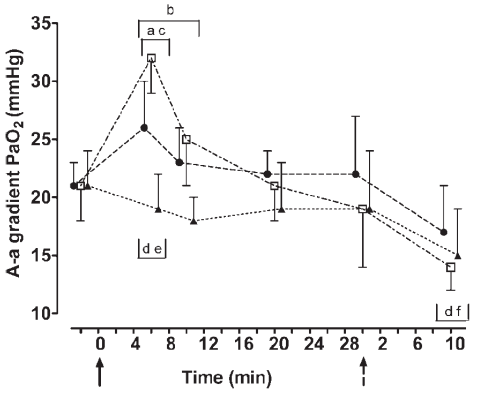


Fig. 5. Drug effects on alveolar-arterial oxygen partial pressure gradient (A-a gradient). Values are A-a gradient (means, SD, $n = 8$) of goats injected (solid arrow, time = 0 min) with (intramuscular + intravenous) etorphine + water (□), etorphine + zacopride (●), and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30 min) indicates intravenous injection of diprenorphine. [a]$P < 0.05$, etorphine + zacopride vs. etorphine + water; [b]$P < 0.05$, etorphine + 8-OH-DPAT vs. etorphine + water; [c]$P < 0.05$, etorphine +zacopride vs. etorphine + 8-OH-DPAT; [d]$P < 0.05$, etorphine + water preinjection vs. postinjection/reversal; [e]$P < 0.05$, etorphine + zacopride preinjection vs. postinjection; and [f]$P < 0.05$, etorphine + 8-OH-DPAT preinjection vs. postreversal (2-way ANOVA with post hoc SNK test). A-a gradients were not significantly different among the trials before the agents were injected [$F_{(10,70)} = 8.23$, $P > 0.05$].

the gradient remained below the preinjection gradient throughout immobilization. Coadministration of zacopride attenuated [$F_{(10,70)} = 8.23$, $P = 0.003$] but did not abolish the effects of etorphine on the A-a gradient [$F_{(10,70)} = 8.23$, $P < 0.002$]. After administration of diprenorphine, the A-a gradients dropped significantly below preinjection values in the etorphine + water [$F_{(10,70)} = 8.23$, $P = 0.0004$] and the 8-OH-DPAT + etorphine [$F_{(10,70)} = 8.23$, $P = 0.005$] trials.

Heart rate. Figure 6 shows the effects of administrating etorphine, with and without serotonergic ligands, on heart rate. Heart rate was 67 (SD 5) beats/min ($n = 8$) before etorphine administration. Over the time course of the immobilization, heart rate decreased after etorphine administration, whether or not the ligands were coadministered. In contrast to its effect on respiratory variables, etorphine administration did not affect heart rate immediately. Heart rate was unchanged for at least the first 8 min after etorphine administration. Thereafter, the decline in heart rate was attenuated by coadministration of zacopride but accentuated by coadministration of 8-OH-DPAT. After the second 10-min interval, heart rate was significantly [$F_{(2,7)} = 0.33$, $P < 0.001$] decreased after 8-OH-DPAT coadministration and increased [$F_{(2,7)} = 0.33$, $P < 0.01$] after zacopride coadministration, compared with heart rate following coadministration of etorphine with water. In the etorphine + water and etorphine + zacopride trials, heart rates returned to the preinjection rates after diprenorphine administration, whereas heart rate in the etorphine + 8-OH-DPAT trial remained significantly (Student's paired *t*-test, $P < 0.0001$) lower than the preinjection rate.

Mean arterial pressure. Figure 7 shows the effect of administration of etorphine, with and without the serotonergic ligands, on mean arterial pressure. Mean arterial pressure before the administration of etorphine was 108 (SD 12) mmHg ($n = 8$). Etorphine administration had a biphasic effect on the mean arterial pressure. For the first 6 min after etorphine + water administration, mean arterial pressure increased, and then it gradually decreased throughout the immobilization period. Coadministration of 8-OH-DPAT with etorphine enhanced the biphasic pressure changes. In the first 10-min interval, mean arterial pressure after coadministration of 8-OH-DPAT with etorphine was significantly ($F = 0.94$, $P = 0.0015$) higher than that following etorphine + water and etorphine + zacopride. Zacopride coadministration attenuated the biphasic effects of etorphine administration and significantly (Student's paired *t*-test, $P = 0.025$) reduced mean arterial pressure throughout the immobilization. After the administration of diprenorphine, mean arterial pressures were significantly higher than preinjection values in the etorphine + water (Student's paired *t*-test, $P = 0.0004$) and etorphine + 8-OH-DPAT (Student's paired *t*-test, $P = 0.01$) trials. After the administration of diprenorphine, mean arterial pressure was significantly (Student's paired *t*-test, $P = 0.007$) lower than preinjection pressure in the etorphine + zacopride trial.

DISCUSSION

At a dose at which it immobilized goats, the opioid etorphine caused marked respiratory depression. Symptomatically, this depression was evident as a decrease in respiratory rate to about one-half the rate before etorphine administration. The respiratory rate remained low throughout the immobilization period, but, alone, it did not reveal the respiratory status of the animals. Directly after the administration of etorphine and up until 10 min after injection, respiratory depression was the most severe; the animals became clinically hypoxic, taken as $Pa_{O_2} < 60$ mmHg and percent arterial hemoglobin saturation

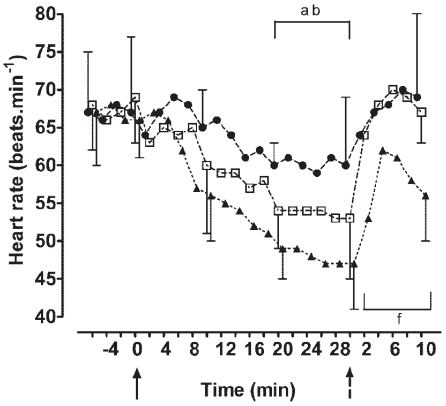


Fig. 6. Drug effects on heart rate. Values are heart rate (means, SD, $n = 8$) of goats injected (solid arrow, time = 0 min) with (intramuscular + intravenous) etorphine + water (□), etorphine + zacopride (●), and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30 min) indicates intravenous injection of diprenorphine. [a]$P < 0.0125$, etorphine + zacopride vs. etorphine + water; and [b]$P < 0.0125$, etorphine + 8-OH-DPAT vs. etorphine + water (1-way ANOVA with post hoc SNK test on areas between the curves). [f]$P < 0.025$, etorphine + 8-OH-DPAT preinjection vs. postreversal (Student's paired *t*-test). Heart rates were not significantly different among the trials before the agents were injected ($F = 0.03$, $P = 0.7$).

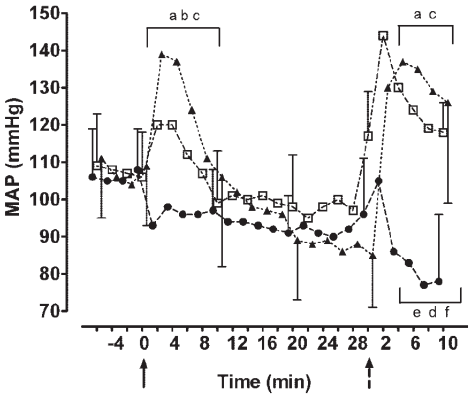


Fig. 7. Drug effects on mean arterial pressure. Values are mean arterial pressure (means, SD, $n = 8$) of goats injected (solid arrow, time = 0 min) with (intramuscular + intravenous) etorphine + water (□), etorphine + zacopride (●), and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30 min) indicates intravenous injection of diprenorphine. [a]$P < 0.0125$, etorphine + zacopride vs. etorphine + water; [b]$P < 0.0125$, etorphine + 8-OH-DPAT vs. etorphine + water; and [c]$P < 0.0125$, zacopride + etorphine vs. 8-OH-DPAT + etorphine (1-way ANOVA with post hoc SNK test on areas between the curves). [d]$P < 0.025$, etorphine + water preinjection vs. postreversal; [e]$P < 0.025$, etorphine + zacopride preinjection vs. postreversal; and [f]$P < 0.025$, etorphine + 8-OH-DPAT preinjection vs. postreversal (Student's paired t-test). Mean arterial pressure values were not significantly different among the trials before the agents were injected ($F = 0.41$, $P = 0.67$).

<85% (8). Hypoxia resulted from both a decrease in the ventilation, indicated by an increase in Pa_{CO_2}, and a decrease in diffusion, presumably via a ventilation-perfusion mismatch, indicated by an increase in the A-a gradient in oxygen partial pressure. After 10 min, there was a gradual increase in both the Pa_{O_2} and the percent oxygen hemoglobin saturation values, which was brought about predominantly by an improvement in diffusion (compare Figs. 4 and 5).

That opioids depress respiration is well known. What we have shown, we believe for the first time, is that the depressed respiratory function can be reversed substantially by administration of serotonergic ligands. Coadministration of zacopride or 8-OH-DPAT with etorphine improved the respiratory function of the goats such that Pa_{O_2} and arterial hemoglobin saturation remained above levels defining clinical hypoxia. The ligands, which act at different 5-HT receptors, reversed respiratory depression via different physiological mechanisms. Zacopride attenuated the decrease in the respiratory rate and decreased the hypercapnia, indicating improved ventilation. 8-OH-DPAT also attenuated the decrease in the respiratory rate but did not improve ventilation, because Pa_{CO_2} remained elevated (Fig. 4). The main beneficial effect of 8-OH-DPAT was on the pulmonary circulation; it improved diffusion, as indicated by the restoration of normal differences between alveolar and arterial partial pressures of oxygen (Fig. 5), presumably by improving ventilation-perfusion ratios. Zacopride also partially restored the A-a gradient, but its effect was not as great as that of 8-OH-DPAT. In addition to the deleterious effects on the respiratory system, the opioid also affected the cardiovascular status of the goats by inducing bradycardia and transient hypertension (Figs. 6 and 7). The serotonergic ligands influenced those cardiovascular effects, too. Zacopride abolished the hypertension, whereas 8-OH-DPAT transiently exacerbated the etorphine-induced biphasic changes in mean arterial pressure (Fig. 7). Similarly, zacopride reduced, and 8-OH-DPAT enhanced, the bradycardia (Fig. 6).

Both serotonergic ligands improved respiratory function and affected the cardiovascular status without reversing catatonic immobilization, a necessity given that the primary use of etorphine is chemical immobilization of animals. Indeed, coadministration of both zacopride and 8-OH-DPAT with etorphine significantly decreased the time it took for the goats to become recumbent. Thus we have shown that the serotonergic ligands zacopride and 8-OH-DPAT, acting through physiologically distinct mechanisms, improved the respiratory status of goats immobilized with the opioid etorphine, without reversing catatonic immobilization, and zacopride also improved the cardiovascular status of the goats.

It should be noted that the laboratory in which we conducted our experiments was situated at an altitude at which the respiratory status of even intact animals is somewhat different from that at sea level; Pa_{O_2}, for example, was 70 ± 4 mmHg in the goats before immobilization. However, we have no reason to suspect that the effects of the agents on the respiratory system would differ at altitudes lower than ours, although actual values of variables like the partial pressure of blood gases and the oxygen hemoglobin saturation would differ. Another potential limitation of our study is that zacopride and 8-OH-DPAT are ligands that act on more than one serotonin receptor. Where we have drawn conclusions about the effects of zacopride or 8-OH-DPAT on one specific receptor, we have based these conclusions on the results from previous studies that have investigated the function of specific 5-HT receptor ligands.

Serotonergic receptors in neuronal pathways play important roles in the modulation of respiratory rhythm (33). Many studies have examined the effects of serotonin and its congeners on the function of respiratory neurons, specifically during sedative-induced compromise of those neurons. Indeed, the actions of the ligands that we employed have been explored in that context. Sahibzada et al. (37) showed that 8-OH-DPAT reversed the morphine-induced suppression of neuronal activity in anesthetized rats, and Lalley et al. (22) used 8-OH-DPAT to reverse pentobarbital- and ketamine-induced suppression of respiratory neurons in cats. Richter et al. (33) claimed that the effect of 8-OH-DPAT on the neurons generating respiratory rhythm results from its agonism of 5-HT_7 receptors. They proposed that the reversal of morphine-induced neuronal suppression observed by Sahibzada et al. (37) depended on 8-OH-DPAT's action on 5-HT_7 receptors and not, as Sahibzada et al. had believed, on 5-HT_{1A} receptors. Even if the action of 8-OH-DPAT is mediated by the 5-HT_7 receptors, 5-HT_{1A} receptors also are facilitatory in reversing morphine-induced suppression of respiratory neurons, because buspirone, a 5-HT_{1A} agonist that has no effect on the 5-HT_7 receptor (33), also reversed the suppression (37). 8-OH-DPAT may well improve the activity of the neurons generating respiratory rhythm through its action on both the 5-HT_{1A} and 5-HT_7 receptors. We believe that 8-OH-DPAT increased respiratory frequency in our goats through its action on respiratory neurons, rather than through the enhancement of the hypoxic drive that the goats experienced after etorphine administration. This belief is supported by the finding that 8-OH-DPAT did not

increase respiratory frequency or ventilation rate in hypoxic goats (20).

8-OH-DPAT's activation of 5-HT_7 receptors provokes c-AMP formation (33) in respiratory neurons, which then stimulates the respiratory rhythm (2). It is not clear how 8-OH-DPAT's concomitant activation of the 5-HT_{1A} receptors could improve respiratory rhythm, although Lalley et al. (22) found that, in anesthetized cats, 8-OH-DPAT's action on 5-HT_{1A} receptors prevented prolonged discharge of early inspiratory neurons. In another study, Lalley et al. (21) showed that the effect of 8-OH-DPAT on inspiratory neurons is dose dependent. At lower doses (10–50 μg/kg), 8-OH-DPAT increased the frequency of phrenic nerve discharges in anesthetized cats, but higher doses (50 and 90 μg/kg) suppressed phrenic nerve discharges. In a similar and more recent study (34), phrenic nerve discharges were decreased even when 20 μg/kg 8-OH-DPAT was injected intravenously in cats. We used a much higher dose (500 μg/kg) of 8-OH-DPAT in our goats, and we did not observe any effects consistent with depression of respiratory neurons. Sahibzada et al. (37) also found that 8-OH-DPAT had no depressant effects on rat respiratory neurons when injected at a dose of 100 μg/kg.

In contrast to the uncertainties about the action of 8-OH-DPAT on respiratory neurons, the action of zacopride on such neurons seems to derive unambiguously from its agonism of 5-HT_4 receptors, rather than antagonism of 5-HT_3 receptors. Zacopride has been shown to be an agonist of the 5-HT_{4a} receptor isoform (28), and Manzke et al. (25) discovered that inspiratory neurons in the pre-Bötzinger complex host both 5-HT_{4a} and μ-opioid receptors. Stimulation of the μ-opioid receptors would decrease cAMP in inspiratory neurons (2) and consequently decrease inspiratory drive, whereas stimulation of the 5-HT_{4a} receptors would increase cAMP and thus increase inspiratory drive (25).

In contrast to the degree of investigation on the actions of serotonergic ligands on respiratory neurons, as far as we can establish, no one has investigated the actions of serotonergic ligands on the function of the effecter organs in the respiratory system. It is far from obvious how activity on neurons responsible for respiratory rhythms would translate into effects on the clinically important phenomena of hypoxia and hypercapnia induced by opioids, nor, as we think we have discovered, is it guaranteed that improvement of oxygenation results from actions on respiratory neurons. We have demonstrated that, in goats subjected to opioid immobilization, although 8-OH-DPAT improved respiratory rate, it did not improve alveolar ventilation; hypercapnia did not decrease when 8-OH-DPAT was coadministered with etorphine. Nevertheless, 8-OH-DPAT coadministered did improve Pa_{O_2}. We believe that this increase in Pa_{O_2} depended on 8-OH-DPAT countering the effects of the opioid on the pulmonary vasculature. Opioids decrease Pa_{O_2}, both by reducing alveolar ventilation and by disrupting pulmonary blood perfusion. Pulmonary perfusion decreases under the influence of opioids both because hypoxia causes pulmonary vasoconstriction (31) and because opioids directly cause pulmonary vasoconstriction (19, 38). They do so by inducing the release of histamine in the lungs (17, 26) and by activating the sympathetic nervous system centrally (36). We believe that 8-OH-DPAT improved blood oxygenation primarily by reducing pulmonary blood shunting, through its serotonergic effects on the pulmonary vasculature.

Serotonin has a strong vasoactive effect on the pulmonary vasculature (15). In goats, serotonin causes vasoconstriction in the pulmonary arteries and vasodilation in the pulmonary veins (10). Serotonin-induced pulmonary vasoconstriction appears to be brought about mainly by the activation of 5-HT_{2A} receptors (24), to which our ligands did not bind, and pulmonary venodilation by the activation of 5-HT_4 receptors (11). Although no one appears to have explored the effects of 5-HT_7 receptor activation in the goat's pulmonary vasculature, we believe that 8-OH-DPAT may have improved the pulmonary perfusion that had been compromised by opioid administration, through its action on 5-HT_7 receptors. Our belief is supported by the identification of 5-HT_7 receptors in the pulmonary vasculature of many other mammalian species (4, 42) and the observation that 5-HT_7 receptor activation causes smooth muscle relaxation (42, 43). There also is evidence that 5-HT_7 receptors may be involved in pulmonary vasodilation in rabbits (30).

Zacopride causes venodilation in the pulmonary vasculature through its action on 5-HT_4 receptors (11). Venodilation would increase pulmonary perfusion, and although any increase in pulmonary perfusion would have contributed to improving oxygenation, in our goats zacopride acted primarily to improve ventilation, in so doing, reducing hypercapnia and improving both Pa_{O_2} and hemoglobin oxygen saturation. It seems likely that the activity of zacopride on pre-Bötzinger neurons, compromised by opioid administration, accounted for the restoration of ventilation.

Although there have been several studies showing that serotonergic ligands act on respiratory networks in the central nervous system (21–23, 25, 37), we believe that our study is one of the few showing the effects of serotonergics on blood gases and that it is the first study showing that serotonergics reverse opioid-induced respiratory depression and hypoxia without reversing catatonic immobilization, an outcome that mirrors, for the whole animal, the conclusion of Manzke et al. (25) that a serotonergic ligand can excite respiratory neurons without affecting those involved in analgesia. We also have shown that the effect of serotonergics on the pulmonary vasculature plays an important role in influencing respiratory status, in addition to effects mediated by central respiratory networks. In addition to their effects on the pulmonary vasculature, the ligands also affect the general circulation, with zacopride improving the deleterious consequences of the opioid on blood pressure and heart rate and 8-OH-DPAT worsening them, but only mildly and transiently.

Opioids are used in veterinary practice and game management to immobilize mammals (16, 40). They induce a catatonic immobilization by acting on localized areas in the central nervous system (41). In the rat, at least, the most prominent of these areas are the nucleus raphe pontis (1, 5, 6, 41, 44) and the nucleus accumbens (12). Both these nuclei contain serotonergic receptors (44), and serotonin enhances opioid-induced catatonia (6, 12, 44). To the best of our knowledge, no one has identified which serotonin receptors are involved in such enhancement. We have shown that both zacopride and 8-OH-DPAT enhanced opioid-induced catatonia in that both reduced time to recumbency in our goats when coadministered with etorphine. Subsequently, though, zacopride somewhat reduced, rather than enhanced, the sedative effects of etorphine. This finding may be explained if zacopride, through its 5-HT_3 antagonistic effects, reversed the effects of κ-opioid receptors

(18), thereby resulting in a decrease in opioid-induced hypotonic immobility (9). It would seem that more than one serotonergic receptor mediates the enhancement of opioid-induced immobilization, but because these ligands each act on two 5-HT receptor types, we are unable to draw any conclusions as to which receptors are involved. We do know that neither ligand, at least at the dose we used, brought about immobilization in its own right.

We postulate that the key serotonergic receptors involved in combating opioid-induced respiratory depression, at least in goats, are the 5-HT_4 and 5-HT_7 receptors, but positive identification of the receptors will require further studies with specific ligands. However, until we also know which serotonergic receptor is responsible for improving opioid-induced catatonic immobilization, we should not conclude that a specific receptor ligand would be the most putative therapeutic agent to improve both immobilization and respiratory welfare.

In summary, we have shown that the serotonergic ligands improve blood oxygenation in goats with respiration depressed by opioid administration, by improving both ventilation and oxygen diffusion, we believe, by improving pulmonary perfusion. Further studies are required to identify the mechanisms involved and will require measurements of pressures and flows in the respiratory system. Also, although our focus has been on reduction of morbidity and mortality resulting from respiratory depression in animals immobilized by opioid administration and although extrapolation between species should be made with caution, we feel that we also have provided more evidence that serotonergic ligands might be useful in reversing respiratory depression in patients under opioid analgesia or anesthesia, without interfering with the intentional effects of the opioids.

ACKNOWLEDGMENTS

We thank the staff of the Central Animal Services of the University of the Witwatersrand for animal management and support throughout this study. We thank Geoff Salter for developing the processor for monitoring blood pressures. We thank Susan Dantu from Arrow Africa for a donation of intra-arterial catheters. We also thank Linda Fick, Robyn Hetem, Georg von Bormann, Lennox Nqobo, and David Makoa for dedicated support and assistance with experimental procedures.

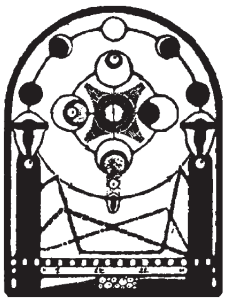
GRANTS

This work was funded by a Faculty Research Committee Grant, University of the Witwatersrand, and by the National Research Foundation, South Africa.

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CHAPTER 5

Effects of serotonin agonists and doxapram on respiratory depression and hypoxemia in etorphine-immobilized impala (*Aepyceros melampus*)

Meyer LCR, Hetem RS, Fick LG, Mitchell D and Fuller A. In press: Journal of Wildlife Diseases

EFFECTS OF SEROTONIN AGONISTS AND DOXAPRAM ON RESPIRATORY DEPRESSION AND HYPOXEMIA IN ETORPHINE-IMMOBILIZED IMPALA (*AEPYCEROS MELAMPUS*)

Leith C. R. Meyer,[1,2] Robyn S. Hetem,[1] Linda G. Fick,[1] Duncan Mitchell,[1] and Andrea Fuller[1]

[1]Brain Function Research Group, School of Physiology, University of the Witwatersrand, 7 York Road, Parktown, 2193, South Africa

[2]Corresponding author (email: Leith.Meyer@wits.ac.za)

ABSTRACT: Respiratory depression is a common side-effect when opioids are used to immobilize wildlife. Serotonergic ligands have the potential to reverse opioid-induced respiratory depression. We therefore examined whether any of three serotonergic ligands could reverse this depression in etorphine-immobilized (0.07mg/kg) impala (*Aepyceros melampus*). The study took place from September to December 2007. Impala received intravenous injections of metoclopramide (10mg/kg, n=6), buspirone (0.05mg/kg, n=8), pimozide (1mg/kg, n=8), doxapram (1mg/kg, n=6), and control solutions, on separate occasions. During the immobilization, partial pressures of oxygen (PaO_2 mmHg) and carbon dioxide ($PaCO_2$ mmHg), respiratory rate (breaths/min), ventilation (l/min), peripheral O_2 saturation (%), tidal volume (l) and the respiratory exchange ratio (RER) were measured before and after the injection of the experimental drugs. Etorphine immobilization caused respiratory depression and hypoxia (mean±SD, $PaCO_2$=51±2 mmHg, PaO_2=40±3 mmHg). Metoclopramide and buspirone, but not pimozide, attenuated the hypoxic effects of etorphine; at 3 min after injection, metoclopramide increased the PaO_2 by 7.5±6.3 mmHg and buspirone by 6±6.6 mmHg (F=3.9; P=0.02). These effects were similar to those of doxapram (8±7 mmHg, F=3.9; P>0.05). Neither metoclopramide nor buspirone significantly increased ventilation but they increased PaO_2 by significantly improving the alveolar-arterial oxygen partial pressure gradient (A-a gradient, F=1.4; P<0.05), indicating an improvement in oxygen diffusion. Metoclopramide and buspirone transiently improved the blood oxygenation of opioid-immobilized impala, probably by improving ventilation-perfusion ratios, without reversing catatonic immobilization.

Key words: Buspirone, doxapram, hypoxia, metoclopramide, opioid, pimozide, respiratory depression.

INTRODUCTION

Potent opioid drugs are commonly used to chemically immobilize wild animals (Swan, 1993; Meltzer and Kock, 2006). However, their use for this purpose comes at a cost to breathing and tissue oxygenation. Opioid receptor antagonists can counteract the depressant effects of opioids on respiration, but they block analgesia, and reverse anaesthesia, sedation and chemical immobilization (Bowdle, 1988; Swan, 1993). Other drugs that have been used to clinically reverse opioid-induced respiratory depression, like bemegride, nikethamide and picrotoxin, have lost favour because of their convulsogenic effects, or because they cause unwanted central nervous system arousal (Bowdle, 1988; Swan, 1993). Thus novel pharmacological approaches are needed to reverse opioid-induced respiratory depression while preserving the therapeutic value of opioids, particularly as analgesic and immobilizing agents.

One such novel approach is the use of serotonergic ligands. The potential of serotonergic ligands to reverse opioid-induced respiratory depression, without reversing the analgesic or immobilizing effects of the opioids, has been demonstrated in several studies (Sahibzada et al., 2000; Manzke et al., 2003; Richter et al., 2003; Meyer et al., 2006; Wang et al., 2007; Guenther et al., 2009) In opioid-immobilized goats that received serotonergic agonists, oxygen partial pressures improved without a change in ventilation (Meyer et al., 2006); this improvement was assumed to occur by the direct action of the agonists on pulmonary vessel diameter, which would improve lung perfusion. We therefore investigated whether serotonergic agonists, currently commercially available, reverse opioid-induced respiratory depression in animals, as indicated by arterial blood oxygenation, without deleterious effects on the target action of the opioids.

From the repertoire of serotonergic drugs already available for human and animal use, experimental drugs were selected for their effects on 5-HT_4, 5-HT_{1A} and 5-HT_7 serotonin

receptors, and consequently, their role in the maintenance of respiratory rhythm (Richter et al., 2003). Of these drugs metoclopramide was selected for its 5-HT_4 receptor agonist effects (Langlois and Fischmeister, 2003; Rang et al., 2003), buspirone for its 5-HT_{1A} receptor agonist effects (Rang et al., 2003) and pimozide for its 5-HT_7 receptor agonist effects (Roth et al., 1994). The effects of these experimental drugs were compared to those of doxapram, an analeptic still used commonly to reverse opioid-induced respiratory depression (Bowdle, 1988), particularly during chemical immobilization (Swan, 1993). Impala (*Aepyceros melampus*) were chosen as the experimental animals, because they are known to be particularly susceptible to the respiratory depressant effects of opioids during chemical immobilization (Burroughs et al., 2006) and because they are captured frequently for African wildlife management. The hypothesis was that all three of the selected serotonergic drugs would attenuate opioid-induced respiratory depression and hypoxia in chemically immobilized impala.

MATERIALS AND METHODS

The procedures were approved by the University of the Witwatersrand's Animal Ethics Screening Committee (clearance number 2005/80/05). The study took place from September to December 2007 at the Lichtenburg Game Breeding Centre (26º07'S, 26º07'E) of the South African National Zoological Gardens, 220 km west of Johannesburg, South Africa.

Animals

Nineteen healthy adult male (n=2) and female (n=17) impala (*Aepyceros melampus*), weighing 36±6 kg (mean±SD), were used. The impala were caught from the wild and transported to bomas (holding pens with solid wooden pole walls) where they were housed for three months

before the experimental trials. Throughout the trials, the impala were housed in 10 m x 5 m bomas, with a maximum of five impala per boma. The impala received lucerne/alfalfa (*Medicago sativa*) and water *ad libitum* and the bomas were cleaned regularly, so the impala were accustomed to occasional human presence.

Drugs

For each trial, we injected etorphine intramuscularly (I.M.), using a dart (see Table 1 for details of the drugs used). On separate days, the experimental drugs and their controls were injected as intravenous (I.V. into the jugular vein following placement confirmation by withdrawing an adequate volume of blood into the syringe) boluses into immobilized animals. Doxapram was injected at a mid-range dose recommended by the manufacturers. The doses of metoclopramide, buspirone, and pimozide were established in a pilot dose-response study, in a separate group of impala (n=15), as mid-range doses which improved respiratory rate or arterial blood oxygenation. As controls, the vehicles, injectable water and DMSO, were injected on their own, at the volumes used to dissolve the experimental drugs. The animals received only two of the experimental drugs and one or both of the controls, on separate occasions. Animals were darted a maximum of four times only and, if they were not fully immobilized or clinically unfit on one of these occasions, experimental procedures were not performed. The drugs and their controls were used randomly across the group of animals; ten animals received water, eight buspirone, six metoclopramide, six doxapram, eight pimozide and five DMSO.

TABLE 1. Agents used during the experimental trials

Agent	Manufacturer	Dose mg/kg	Concentration mg/ml	Vehicle	Known receptor activity		References
					Primary	Secondary	
Etorphine hydrochloride	Novartis, Johannesburg, South Africa	0.071	9.8	water	μ-opioid agonist	κ & σ-opioid agonist	(Roquebert and Delgoulet, 1988)
Doxapram hydrochloride	Intramed, Port Elizabeth, South Africa	1	20	water	peripheral chemoreceptors	central effects (mechanism not known)	(Nishino et al., 1982; Martindale, 1999)
Metoclopramide Hydrochloride	Sigma-Aldrich, Aston Manor, South Africa	10	500	water	5-HT_4 agonist D_2 antagonist	5-HT_3 antagonist	(Martindale, 1999; Langlois and Fischmeister, 2003; Rang et al., 2003)
Buspirone hydrochloride	Sigma-Aldrich	0.05	5	water	5-HT_{1A} agonist	dopamine receptor dependant effects	(Riblet et al., 1982; Taylor, 1988; Martindale, 1999; Rang et al., 2003)
Pimozide	Sigma-Aldrich	1	15	DMSO	5-HT_7 agonist D_2 antagonist		(Roth et al., 1994; Rang et al., 2003)

Experimental procedure

Each impala was immobilized with etorphine hydrochloride on three or four occasions, separated by at least 72 hours, by darting them with a dart gun (Sabi 500, SABI Werkswinkel t/a Magnum Arms, Nelspruit, South Africa; Pneu-Dart dart type P, 3 ml volume, 25 mm long, wire-barbed needle, Pneu-Dart, Williamsport, USA). With the use of a powder charge the darts were fired over a 4-5 m distance into the gluteus muscles. Once an impala was fully immobilized and became recumbent, it was moved to a shaded boma, where it was placed on a veterinary scale (Associated Scale Corporation, Johannesburg, South Africa) and held in a sternal recumbency. The impala was blindfolded and cotton wool was placed in its ears, to reduce external sensory stimuli.

The impala's ear was shaved and swabbed with 5 % chlorhexidine gluconate (Hibitane, Astra Zeneca, Johannesburg, South Africa) in 100 % ethanol, and a 22 gauge intravenous catheter (Introcan, B/Braun, Melsungen, Germany) was placed in one of the auricular arteries. To ensure continuous measures of peripheral haemoglobin oxygen saturation, two veterinary pulse oximeters (Nonin 9847V, Nonin Medical, North Plymouth, USA) were clipped (lingual sensor 2000SL) to the wall of the vulva in female impala, or to a tented skin fold in the perineum of male impala, and on the skin (2000T animal transflectance sensor) at the tail base (secured with adhesive tape). Rectal temperatures were measured with a thermocouple thermometer (BAT-12, Physitemp, Clifton, USA) and were used as the body temperature needed to calculate water vapour pressure in alveolar air and to correct for blood gas measurements. A clear canine anaesthetic face mask (J-298C, Jorgensen Laboratories, Loveland, USA) was placed over the muzzle of the impala, positioned so as to limit the dead space, and a gasket made from a latex glove formed a tight seal between the

impala's muzzle and the face mask. The face mask was connected to a two-way valve which directed all the expired air into a PowerLab Exercise Physiology System (ML870B80, ADIntruments, Castle Hill, Australia). This system consisted of a respiratory flow head (MLT1000L) linked to a spirometer (ML140) and a gas mixing chamber (MLA245) from which the expired gas temperature was measured and mixed gas was sampled through a gas analyser (ML205). The data from these components was collected via the PowerLab 8/30 amplifier (ML870) and integrated with the Metabolic Module software. The simultaneous measurement of respiratory gas concentrations and air flow allowed for the calculation of (at BTPS - body temperature and pressure saturated) minute ventilation (l/min), oxygen consumption (VO_2, l/min), and carbon dioxide production (VCO_2, l/min) and the respiratory exchange ratio (RER = VCO_2/VO_2). Respiratory rate and tidal volume also were measured. Each day the spirometer was calibrated using a 3l calibration syringe and the gas analyzer with a calibration gas (12% O_2, 5.1% CO_2 Air Products, Johannesburg, South Africa) and inspired air (20.93% O_2, 0.03% CO_2).

Once impala were instrumented, which took ~ 8 min (490±116 s, mean±SD) from the time the impala became recumbent after darting, data recordings started. Data were recorded for 4 min before injection (injection time = 0 min) of the experimental drugs or controls, and for 18 min after injection. A 0.5 ml auricular arterial blood sample was drawn 2 min before, and at 3, 10, and 18 min after the injection (these time intervals were selected a priori); the catheter was flushed with 5 iu/ml heparinized (Heparin, Intramed, Johannesburg, South Africa) saline. Directly after each sample was drawn, we measured the arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) using a pre-calibrated blood gas analyzer with pre-calibrated blood gas cassettes (Roche OPTI CCA Analyzer + OPTI cassette B,

Kat Medical, Johannesburg, South Africa). Arterial samples were corrected for body temperature.

During the data recording period, the impala's movements were recorded and assigned a score (see Table 2). At the end of the recording period, all instruments were removed and the impala were returned to their bomas, where the action of etorphine was reversed by injection of 0.096 mg/kg diprenorphine hydrochloride I.V. (M5050, Novartis, Johannesburg, South Africa). A digital stop-watch was used to record the time that it took for the impala to stand after reversal.

TABLE 2. Description used to score the movements of the impala during the immobilization

Movement score	Description
0	no movement
1	movement of the ear or tail
2	movement of the ear and tail
3	movement of the leg or head (with or without the ear or tail)
4	movement of the leg and head (with or without the ear or tail)
5	whole body movement with no attempt to stand
6	whole body movement with an attempt to stand

Ambient dry-bulb temperature and relative humidity were recorded by a data logger (Hobo H08-007-02, Onset Computer Corporation, Pocasset, MA, USA) which was placed in the boma where the experiment took place; ambient temperature ranged between 18 ˚C and 34 ˚C and water vapour pressure between 4.5 and 40.5 mmHg. Barometric pressure, which ranged from 642 mmHg to 650 mmHg, was measured by the on-board barometer of the blood gas analyzer, which we had calibrated against a Fortin mercury barometer (On, F.D & Co Ltd, United Kingdom).

Data analysis

We used GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, USA) for statistical analyses. The alveolar-arterial oxygen partial pressure gradient (A-a gradient) was calculated for an open system (constant pressure) from the formula: FiO_2 (Pb – PH_2O) – $PaCO_2$ – PaO_2 (Dill and Penrod, 1948), where FiO_2 is the fractional inspired oxygen (0.209), Pb the measured barometric pressure (mmHg) and PH_2O the water vapour pressure of saturated air in the alveoli. PH_2O (mmHg) was calculated as 4.58 e{(17.27Tb)/(237.3 + Tb)}(Barenbrug, 1974), where Tb is the body temperature. We assumed that the partial pressure of CO_2 in the alveoli was equal to the arterial partial pressure of CO_2 (Riley et al., 1946; D' Alonzo and Dantzker, 1983; Lumb, 2005).

The change in PaO_2, $PaCO_2$ and A-a gradient were calculated by subtracting post-injection values from the values measured at 2 min before the injection of the agents and controls, while changes in respiratory rate, minute ventilation, tidal volume, peripheral haemoglobin oxygen saturation (mean from the two measurement sites) and median movements were calculated by subtracting post-injection values from the average value measured 4 min pre-injection. Areas under the curves, depicting a change in response over time, were calculated for between 1-3, 4-10 and 11-18 min post-injection (these time intervals were selected a priori), for minute ventilation, respiratory rate, tidal volume, peripheral haemoglobin oxygen saturation, respiratory exchange ratio (RER) and median movements. For parametric data a one-way analysis of variance (ANOVA) followed by a Student Neuman Keuls (SNK) post-hoc test was used to test for differences in the changes in blood gas variables, A-a gradients, time to recumbency, time to standing, and also for differences in areas under the curves, between responses to injectable water, doxapram, metoclopramide

and buspirone and for differences between responses to injectable water, DMSO and pimozide. For non-parametric data a Kruskal-Wallis test with post-hoc Dunn's multiple comparison test was used to test for these differences in the areas under the curves for median movements. To determine if metoclopramide or buspirone effects were different to those of the known respiratory stimulant doxapram within the first 3 min after injection, a Student's unpaired *t*-test was used for all the parametric variables, except for changes in movement (non-parametric data), where a Mann-Whitney test was used. All results are reported as mean±SD, and a $P<0.05$ was considered statistically significant.

RESULTS

Effects of etorphine

Etorphine (0.071±0.005 mg/kg) induced immobilization 223±86 s after darting. The time from darting to immobilization (time to recumbency) did not differ between the trial days ($F=0.54$, $P=0.66$), hence we assumed there to be no accumulative effects of using etorphine repetitively at a 72 h interval. The impala remained sedated and recumbent for the duration (22 min) of each trial. At the start of our recording period, 8-10 min after the impala became recumbent, peripheral haemoglobin oxygen saturation was 76±3 %, PaO_2 40±3 mmHg, $PaCO_2$ 51±2 mmHg, A-a gradient 34±1 mmHg, minute ventilation 10.9±0.8 l/min, respiration rate was 13±1 breaths.min^{-1}, tidal volume 0.8±0.1 l, and the impala were in a state of anaerobic metabolism with RER = 1.04±0.04; these values did not differ across the trials ($P>0.22$). The rectal temperatures at the start of recording were 39.8±3 C and at the end 39.4±3 C, and the profile of the change in temperature was the same for all the trials.

After injection of water, peripheral haemoglobin oxygen saturation increased gradually by 6±7 % (Fig. 3A), PaO_2 by 5.3±5 mmHg (Fig. 1A) and RER by 0.09±0.01 over 18 min. These changes in peripheral haemoglobin oxygen saturation and PaO_2 appeared not to be associated with the effects of an increase in respiratory rate, minute ventilation or tidal volume (Fig. 2), but rather to an improvement in the A-a gradients with time (Fig. 1C).

Effects of doxapram

When doxapram was injected, after etorphine immobilization, PaO_2 increased by 8±7 mmHg (Fig. 1A) and peripheral haemoglobin oxygen saturation increased by 9±9 % (Fig. 3A) by 3 min and remained at similar levels at 10 and 18 min after injection. Compared to water, the increases in PaO_2 and peripheral haemoglobin oxygen saturation were significant only in the first 3 min after the injection of doxapram (PaO_2 F=3.9, P<0.05, peripheral haemoglobin oxygen saturation F=4.2, P<0.05). Increases of PaO_2 and peripheral haemoglobin oxygen saturation were accompanied by both an improvement in the A-a gradient (Fig. 1C) and an acute increase in minute ventilation (Fig. 2B); in the first 3 min after injection, both the A-a gradient (F=4.2, P<0.05) and minute ventilation (F=3.1, P<0.05) were increased compared to water. Although the increase in minute ventilation was significant, doxapram did not decrease the $PaCO_2$ (F=2.1, P>0.05) within the first 3 min or thereafter.

Effects of metoclopramide and buspirone

Compared to water, injections of metoclopramide and buspirone both were followed by increased PaO_2 at 3 min after injection (F=3.9, P<0.05; Fig. 1A);

metoclopramide increased the PaO_2 by 7.5±6.3 mmHg and buspirone by 6±6.6 mmHg. Although PaO_2 increased further at 18 min (metoclopramide 11±6.2 mmHg, buspirone 9.3±9 mmHg), the change in PaO_2 was not different from water at 10 and 18 min (P>0.31). Metoclopramide increased the respiratory rate in the first 3 min after injection (F=3.0, P<0.05; Fig. 2A,). Following metoclopramide and buspirone injection, the increase in PaO_2 was accompanied by improvement in the impala's A-a gradients at 3 min (metoclopramide -5±4 mmHg, and buspirone -3±5 mmHg, F=1.4, P<0.05; Fig. 1C).

Effects of pimozide and DMSO

Compared to water, pimozide did not change the PaO_2 (Fig. 4A), PaCO2, minute ventilation (Fig. 4C), the peripheral haemoglobin oxygen saturation or the A-a gradient, but did increase the respiratory rate in the first 3 min after injection (F=6.8, P<0.05; Fig. 4B,). However, there was no difference between pimozide + DMSO, and DMSO on its own (F=6.8, P>0.05; Fig. 4B). Compared to water, DMSO increased the respiratory rate in the first 3 min after its injection (F=6.8, P<0.01; Fig. 4B).

Comparison of the effects of metoclopramide or buspirone versus doxapram

Metoclopramide's effects on the respiratory variables in the first 3 min after injection were not different to those of doxapram (PaO_2 change P=0.84, $PaCO_2$ change P=0.30, A-a gradient change P=0.57, peripheral haemoglobin oxygen saturation change P=0.25, respiration rate change P=0.32, minute ventilation change P=0.50, change in RER P=0.28), except for tidal volume which was smaller after metoclopramide injection than after doxapram injection (P=0.01). Similarly, in direct comparison to doxapram, buspirone's effects on the respiratory variables in the first 3 min after injection were not different from those of doxapram (PaO_2 change P=0.56,

$PaCO_2$ change P=0.48, A-a gradient change P=0.94, peripheral haemoglobin oxygen saturation change P=0.07, respiratory rate change P=0.65, minute ventilation change P=0.21). In this case there was no difference in the change in tidal volume (P=0.77), but the change in RER was lower (P=0.03) with buspirone than with doxapram.

Effects on immobilization

Metoclopramide caused an increase in movement of impala 4–10 min after its injection (P<0.05; Fig. 3B,). There were no other differences between the movement caused by doxapram, metoclopramide, buspirone and pimozide during any time period.

Effects post-immobilization

Diprenorphine completely reversed the immobilizing effects of etorphine and the impala were standing within 28-59s after its injection. Doxapram, metoclopramide and buspirone did not affect the time to standing (P=0.49), nor did they alter post-immobilization behavior; the impala showed normal coordinated movements, social behavior and normal appetite after the use of these drugs. Earlier injection with DMSO (59±25 s) and pimozide (38±19 s) also did not affect the time to standing (F=2.4, P=0.12). DMSO did not alter post-immobilization behavior, but pimozide caused incessant restlessness and hyperactivity (akathisia), uncontrolled mouth movements (tardive dyskinesia), a stiff gait and a tucked-under stance with muscle tremors (parkinsonism) in some of the animals.

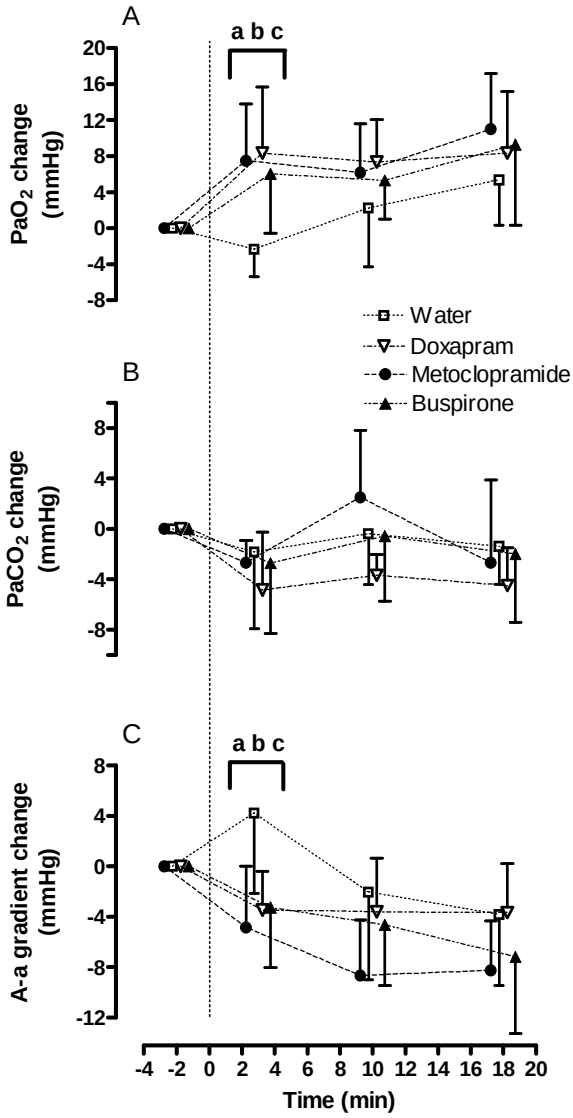


Figure 1. Mean±SD change in arterial partial pressure of oxygen (A), arterial partial pressure of carbon dioxide (B) and alveolar arterial oxygen partial pressure gradient (C), in impala immobilized with etorphine, in response to injection (time = 0) I.V. of injectable water (n=10), doxapram (1 mg/kg, n=6), metoclopramide (10 mg/kg, n=6) and buspirone (0.05 mg/kg, n=8). a, $P<0.05$ doxapram vs. water, b, $P<0.05$ metoclopramide vs. water and c, $P<0.05$ buspirone vs. water (one-way ANOVA with post-hoc SNK test).

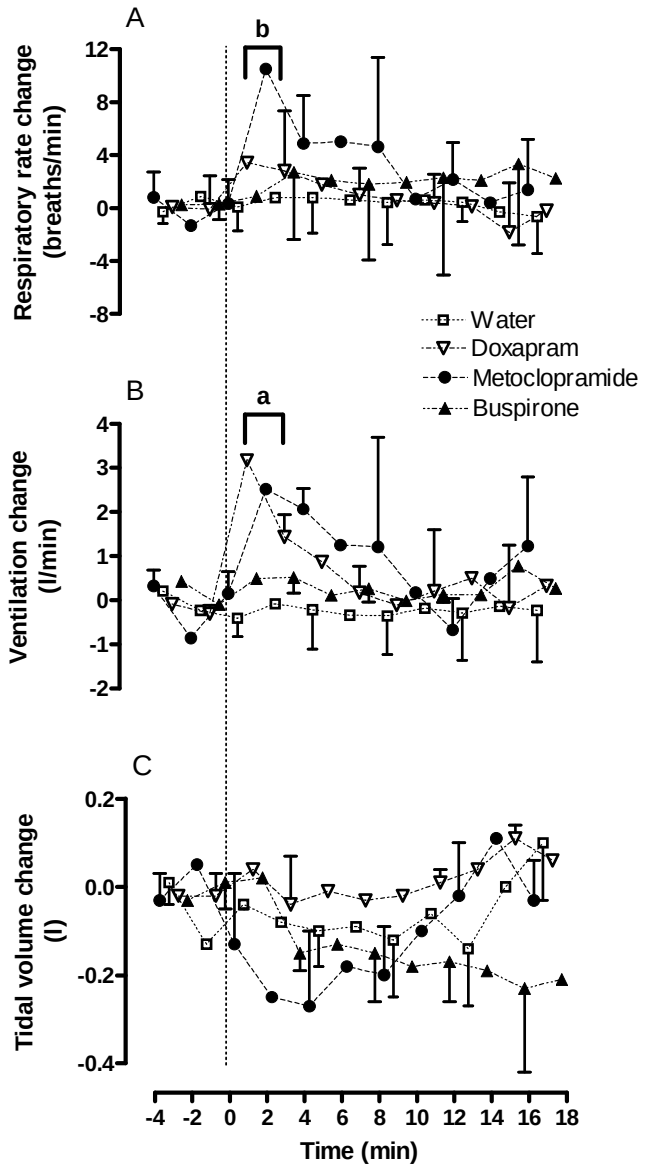


Figure 2. Mean±SD change in respiratory rate (A), ventilation (B) and tidal volume (C), in impala immobilized with etorphine, in response to injection (time = 0) I.V. with injectable water (n=10), doxapram (1 mg/kg, n=6), metoclopramide (10 mg/kg, n=6) and buspirone (0.05 mg/kg, n=). a, $P<0.05$ doxapram vs. water and b, $P<0.05$ metoclopramide vs. water (one-way ANOVA with post-hoc SNK test).

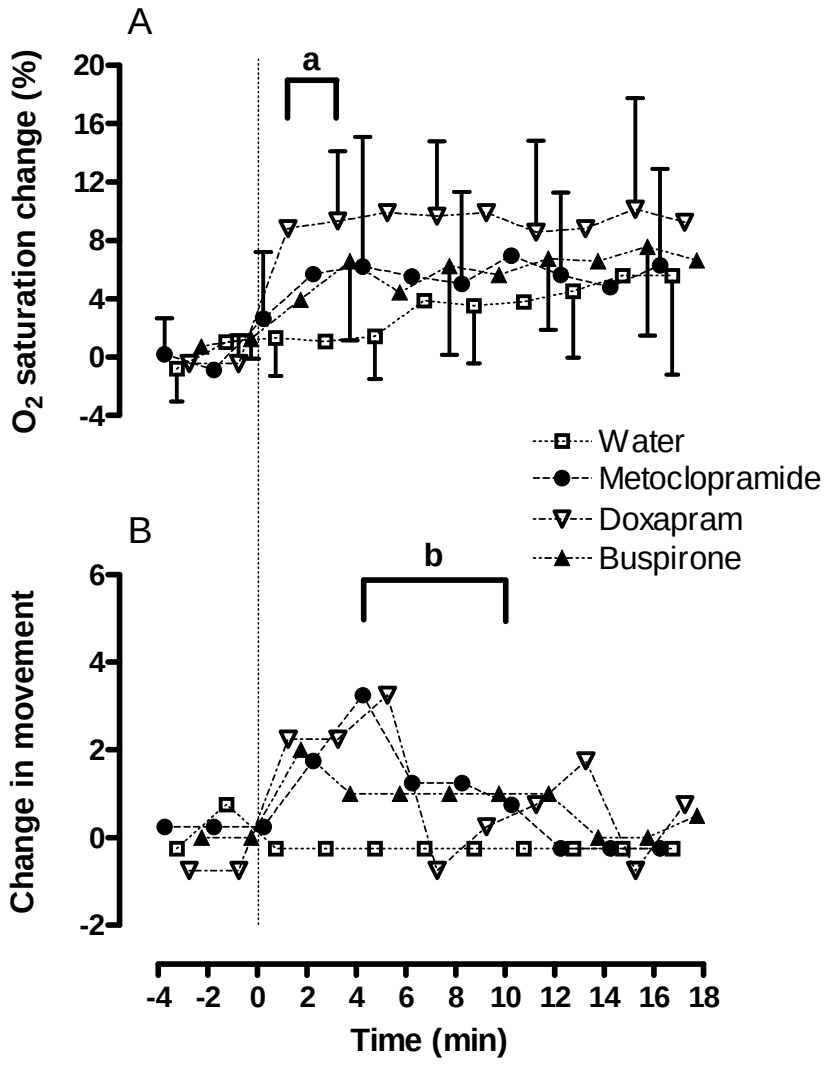


Figure 3. Mean±SD change in the percentage of peripheral haemoglobin oxygen saturation (A) and median movements (B), in impala immobilized with etorphine, in response to injection (time = 0) I.V. with injectable water (n = 10), doxapram (1 $mg.kg^{-1}$, n = 6), metoclopramide (10 mg/kg, n=6) and buspirone (0.05 mg/kg, n=8). a, $P<0.05$ doxapram vs. water and b, $P<0.05$ metoclopramide vs. water (one-way ANOVA with post-hoc SNK test for O_2 saturation changes and Kruskal-Wallis test with post-hoc Dunn's multiple comparison test for changes in movement).

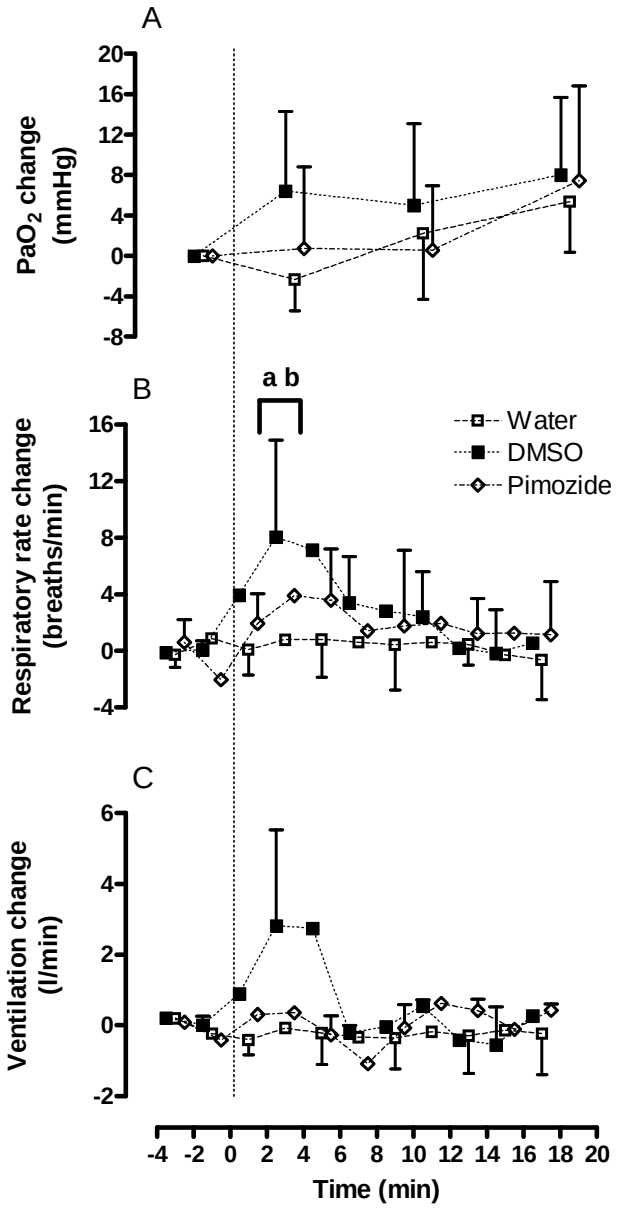


Figure 4. Mean±SD change in arterial partial pressure of oxygen (A), respiratory rate (B) and ventilation (C), in impala immobilized with etorphine, in response to injection (time = 0) I.V. with injectable water (n=10), DMSO (n=5) and pimozide (1 $mg.kg^{-1}$, n=8). a, $P<0.05$ DMSO vs. water and b, $P<0.05$ pimozide vs. water (one-way ANOVA with post-hoc SNK test).

DISCUSSION

We have shown that the opioid-induced hypoxia that occurs during chemical immobilization of a wild antelope can be partially reversed by administration of drugs that have serotonergic actions and which currently are available to veterinarians. At a dose at which it immobilized our impala, the opioid etorphine caused respiratory depression. Hypoxia was the most severe in the first seven minutes of recording (that is, up to 15 min after darting), but the respiratory variables gradually improved over time. However, the animals still remained clinically hypoxic (PaO_2 < 60mmHg, percentage peripheral haemoglobin oxygen saturation < 85%) up until the end of the immobilization. Metoclopramide and buspirone improved the respiratory function of the impala such that PaO_2 levels increased within the first three minutes after injection. These two drugs, which act at different 5-HT receptors, both appeared to attenuate hypoxia by improving oxygen exchange in the lungs, as indicated by the decrease in the differences between alveolar and arterial partial pressures of oxygen (Fig. 1C), presumably by improving ventilation-perfusion ratios. Although neither drug measurably improved minute ventilation, it appeared that metoclopramide did exert some effects on respiratory control, as it initially increased respiratory rate (Fig. 2A). This increase in respiratory rate, however, was accompanied by a decrease in tidal volume, such that minute ventilation did not increase and $PaCO_2$ levels remained elevated (Fig. 2 and 1B).

Like metoclopramide, pimozide also did not improve minute ventilation but it did increase respiratory rate (Fig. 4). However, these effects on respiratory rate did not appear to result from any pharmacological activity of pimozide on central respiratory control, but rather were effects of injecting its solvent DMSO; DMSO

alone increased respiratory rate (Fig. 4B) to a greater extent than when pimozide was dissolved in DMSO.

The respiratory effects of doxapram, the standard drug used by veterinarians to reverse respiratory depression in animals, were similar to those of metoclopramide and buspirone (Fig. 1 and 2). However, doxapram increased minute ventilation over the first three minutes of immobilization, but this increase was not accompanied by a decrease in $PaCO_2$ at 3 min. The disparity may have arisen because the effects of doxapram on minute ventilation were confined to an acute spike that occurred in the first minute after its injection (Fig. 2B). The respiratory effects of metoclopramide and buspirone essentially did not differ to those of doxapram, except that metoclopramide decreased the tidal volume and buspirone led to a smaller increase in the respiratory exchange ratio.

Both metoclopramide and buspirone improved respiratory function in impala immobilized with etorphine without reversing catatonic immobilization, a necessity if the agents are to be used to improve respiration during chemical immobilization of wild animals. However, metoclopramide caused some arousal, evident as more body movement during the immobilization (Fig. 3B). This arousal appeared not to be in phase with the changes in respiratory function (compare Fig. 3B to Fig. 1, 2) and was no different to the arousal and subsequent movements that followed doxapram injection. Thus we have shown that administration of the clinically-available drugs metoclopramide and buspirone, which have serotonergic effects, improved the respiratory status of opioid-immobilized impala, apparently predominantly by improving ventilation-perfusion ratios, without reversing catatonic immobilization.

Buspirone had little effect on the impala's minute ventilation, a finding similar to that seen in humans after oral doses of buspirone (Oertel et al., 2007). However,

buspirone improved arterial blood oxygenation, presumably via an improvement of the ventilation-perfusion ratio, a finding similar to that observed in immobilized goats which received the 5-HT_{1A} agonist 8-OH-DPAT (Meyer et al., 2006). In goats, this effect was believed to be brought about by 8-OH-DPAT's activation of 5-HT_7 receptors (Meyer et al., 2006). However, because buspirone has very little 5-HT_7 activity (Pittalà et al., 2007) we speculate that pulmonary perfusion was improved by decreasing pulmonary sympathetic tone via the activation of 5-HT_{1A} receptors in the central nervous system (Dabiré et al., 1990; Saxena and Villalón, 1990; Zwieten, 1996; Ootsuka and Blessing, 2003). We believe that our impala had high sympathetic activity, and hence pulmonary vasoconstriction (Kadowitz and Hyman, 1973; Lumb, 2005), because of the stress of capture (Meltzer and Kock, 2006), the pharmacological effects of etorphine (Roquebert and Delgoulet, 1988), and the associated hypoxia and hypercapnia caused by etorphine-induced respiratory depression (Heard et al., 1990; Heard et al., 1996). Further evidence that the 5-HT_7 receptors may not be involved in pulmonary perfusion is that the 5-HT_7 agonist pimozide did not improve the A-a gradient or arterial blood oxygenation in our impala. Also, contrary to what we might have expected given the ability of 5-HT_7 activation to restore normal neuronal activity in opioid-induced apnea in rats (Richter et al., 2003), pimozide did not increase respiratory rate compared to its solvent, DMSO. Metoclopramide also increased arterial blood oxygen concentrations apparently also by improving the ventilation-perfusion ratios, and not by improving minute ventilation. Like the beneficial effect of buspirone, that of metoclopramide might be attributable to an increase in pulmonary perfusion, because 5-HT_4 agonists cause potent venodilation in ruminant pulmonary veins (Cocks and Arnold, 1992; Morcillo and Cortijo, 1999).

In summary, we have shown that readily available pharmaceutical agents (metoclopramide and buspirone) with serotonergic actions can be used to improve blood oxygenation in animals with opioid-induced respiratory depression and hypoxaemia. Although these agents did not completely resolve the hypoxaemia, and although there may be other better ways of improving blood oxygenation (i.e., using oxygen insufflation), our findings have a significant potential practical importance considering that these agents, and other more receptor specific serotonin ligands, may be included in a dart where they would have their effects in the period immediately after the animals become recumbent, a period when the respiratory depressant effects of the opioids are most severe and often at a time when a veterinarian is yet to attend to the animal. Our data show that serotonergic ligands can partially improve but cannot eliminate opioid-induced hypoxia, and thus there exists further opportunity to improve respiratory function and blood oxygenation in animals under opioid immobilization. We believe that efforts to do so should focus not only on improving ventilation but also on further improving pulmonary perfusion and ventilation-perfusion ratios.

ACKNOWLEDGEMENTS

We thank the National Zoological Gardens of South Africa for allowing us to access their property and resources, and the staff at the Lichtenburg Game Breeding Centre for their help. We also thank our colleagues from the School of Physiology, University of the Witwatersrand, who helped collect data, in particular Philippa Hidden and Marna Broekman. This work was funded by a Faculty Research Committee Grant, University of the Witwatersrand, and by the National Research Foundation, South Africa.

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CHAPTER 6

Conclusion

Ungulates are genetically programmed to react to any form of danger in a manner that both psychologically and physiologically prepares them to escape. In nature, their danger usually is in the form of a predator attack, and usually exposure to this danger is short-lived, either because it is fatal or an animal escapes. However, during capture procedures not only is the danger novel, and most likely very threatening, but it also is prolonged over a duration that often is longer than natural fleeing times. Therefore, the psychological and physiological reaction to capture may be excessive, and the capture most likely causes one of the most challenging episodes that could occur in a wild ungulate's life.

In the past very crude methods were used to capture wild animals; often these were centred on trapping, handling animals while they were conscious, and chasing animals to exhaustion (Harthoorn 1976; McKenzie 1993). These methods resulted in a high incident of mortalities. Over the past few decades capture procedures have been refined and new techniques have been introduced so as to reduce mortalities. One of the major advances in game capture took place in the 1960's with the introduction and use of highly potent opioid drugs, which induce a rapid reversible catatonic immobilization when small volumes of drugs are used in a dart (McKenzie 1993). This advance allowed for the reduction of fleeing times and reduced the number of animal mortalities during capture operations (Kock et al. 2006; Swan 1993a). However, chemical capture of animals is costly and impractical when large numbers of animals need to be captured. Therefore mass capture techniques for capturing large numbers of animals without the use of capture drugs were developed. Over time these techniques have been advanced and refined, and the introduction of long-acting neuroleptics, which are given to animals post capture, reduced transport and boma

stress and also the incidence of capture myopathy (Ebedes et al. 1996; McKenzie 1993). However, even though these advances have improved capture procedures, and reduced mortality rates, significant morbidity and mortality still occurs. In the research for my thesis, I endeavoured to gain a better understanding of some of the effects of capture on ungulates and to determine whether novel approaches could be used to reduce or reverse some of these capture-related side-effects.

Although the topics of my thesis, capture-induced hyperthermia and opioid-induced respiratory depression, are distinct, both can occur in capture procedures, and both cause a negative oxygen balance which causes an animal to metabolize anaerobically. Because anaerobic metabolism of a prolonged duration may be harmful and lethal to an animal, I focused on improving the understanding of capture-induced hyperthermia, the effects of opioids on respiratory function and pulmonary performance, and the treatment of opioid-induced respiratory depression. Currently, the physiological responses during capture are not well understood because few continuous and accurate measurements of physiological variables have been made. Therefore I focused on accurately measuring body temperature and cardiorespiratory variables before, during and after capture procedures.

The primary cause of capture-induced hyperthermia is not known, and in the past, it has been difficult to determine because it was impossible to continuously measure body temperatures in free-living wild animals before, during and after capture procedures. To better understand the cause of capture-induced hyperthermia I implanted temperature sensitive data loggers which continuously recorded body temperatures in wild impala (Chapter 2 & 3). To determine whether capture drugs

play a role in capture-induced hyperthermia, impala were captured with and without immobilizing drugs (Chapter 2). The role of capture drugs was assessed further in Chapter 3 where different pharmacological combinations of drugs were used to capture impala. In Chapter 2 and 3 environmental conditions were measured to determine whether they influenced capture-induced hyperthermia. In chapter 2 activity levels in both chemically and net captured impala were assessed to determine whether activity plays a role in capture-induced hyperthermia. The role of stress in capture-induced hyperthermia also was assessed in Chapter 2 and 3 by measuring cortisol levels in captured animals. To further assess the role of stress, impala were habituated to boma housing and handling (Chapter 2). Chapter 3 also examined the interaction between chemical immobilization, capture-induced hyperthermia and opioid-induced respiratory depression.

It is known that opioids cause respiratory depression and hypoxia when wild animals are immobilized chemically. These effects have been attributed primarily to a depression in respiratory rate and pulmonary ventilation (Pattinson 2008; Santiago & Edelman 1985), leading to the conclusion that the best treatment of this depression is to partially reverse the opioids' effects or administer drugs that improve ventilation (Meltzer & Kock 2006a; Swan 1993b). However, unlike in humans, very little is known about the effects of opioids on cardiopulmonary performance in wild animals. Furthermore, the reversal agents that currently are used to treat opioid-induced respiratory depression generally also reverse the immobilizing effects of the opioids, and so are unsuitable for use in capture. Recently, there has been a novel suggestion to use serotonergic ligands to reverse opioid-induced respiratory depression. However, this suggestion has been based on the effects of serotonergic ligands on neuronal

function in the respiratory network rather than the effects of these ligands on cardiopulmonary function and performance, and the crucial blood gas variables. To determine whether serotonergic ligands could be used to reverse opioid-induced respiratory depression during chemical immobilization, I used goats in a laboratory setting (Chapter 4). In these goats I measured blood gases and other cardiopulmonary variables to determine the effects of opioids and serotonergic ligands on cardiopulmonary function and performance. Following this study, I conducted a similar study in the field, in wild chemically immobilized impala, to determine if commercially-available serotonergic ligands could be used to reverse opioid-induced respiratory depression (Chapter 5).

The work described in my thesis has yielded important new insights, I believe, into the primary cause of capture-induced hyperthermia, and the role of physical exercise, ambient conditions, and drugs in this hyperthermia. It also has shown that the hypoxia caused by opioids is not caused solely by the depressant effects of opioids on respiratory rhythm and pulmonary ventilation but also, and perhaps more importantly by their effects on pulmonary perfusion and gas exchange. Moreover, I have shown that serotonergic ligands can be used to reverse opioid-induced respiratory depression and that their effects improve not only respiratory rhythm but also oxygen exchange in the lungs.

6.1 Reduction of capture-induced hyperthermia

Wild animals typically develop hyperthermia when captured (Cheney & Hattingh 1987; Gericke et al. 1978; Hofmeyr et al. 1973; Kock et al. 1987a; Kock et al. 1987b; Martucci et al. 1992; Montané et al. 2003; Montané et al. 2007). The nature of this

hyperthermia is that body temperature rises rapidly from the onset of capture and may continue to rise for up to 30 minutes after capture, before declining. Body temperature often exceeds 41-42˚C, the temperature at which cellular damage starts to occur in most mammals (Grint et al. 2007; Haskins 1995). Because of the nature and magnitude of these hyperthermias, the causes and consequences of capture-induced hyperthermia have been equated to those of heat stroke; heat stroke is considered a potential diagnosis when an animal's body temperature has exceeded 40-41˚C (Bouchama & Knochel 2002; Leon 2006; Yan et al. 2006). Heat stroke occurs when the body's normal ability to regulate temperature is overwhelmed by prolonged exposure to heat, with (exertional heat stroke) or without strenuous exercise (classic heat stroke; Bouchama & Knochel 2002; Leon 2006).

Although capture-induced hyperthermia may share some similarities with heat stroke, it occurs through different mechanisms. Capture-induced hyperthermia, for example, occurs irrespective of the environmental conditions, and even when animals are captured in the cold (Montané et al. 2003; Montané et al. 2007). As I have shown (Chapter 2 and 3), the magnitude of capture-induced hyperthermia is not correlated to environmental conditions at the time of capture, a situation different to that prevailing in heat stroke. However, it is likely that environmental conditions play an important role post-capture, as they will determine how easily an animal will lose excessive body heat to the environment. Additionally, immobilizing agents which alter an animal's ability to thermoregulate may result in environmental conditions influencing body temperature.

As for environmental conditions, activity also does not appear to be correlated with the magnitude of capture-induced hyperthermia (Chapter 2), a situation contrary to that of exertional heat stroke. There is no doubt, however, that the metabolic heat that is generated from active muscles will contribute to capture-induced hyperthermia, but its overall contribution may be small (Bakken et al. 1999), especially if exercise duration and intensity is limited during capture procedures. Even if exercise intensity was high, or extended over a long duration, hyperthermia may be small because under natural circumstances animals dissipate heat effectively and rarely experience more than 1-2°C rises in body temperature during exercise. If an animal severely exerts itself, a situation which would not occur naturally unless an animal were to be chased by a predator or captor, it is likely that a large proportion of heat gained could be attributed to muscle activity. However, without the influence of stress, normal heat loss mechanisms would limit the hyperthermia, or an animal would fatigue before body temperatures reach lethal limits (Jessen 2001). Furthermore, we observed precipitous rises in body temperature in captured animals that did not engage in strenuous exercise, and also found that animals that were chased vigorously, but were not captured, had hyperthermias of smaller magnitude than when they were captured (Chapter 2). Therefore, activity also does not appear to be the primary cause of capture-induced hyperthermia.

Previously it has been suggested that capture-induced hyperthermia and capture myopathy are in essence attributable to the same biochemical malfunction as Porcine Stress Syndrome (Mitchell & Heffron 1982). Although pigs that suffer from Porcine Stress Syndrome develop an acute precipitous rise in body temperature similar to that of capture-induced hyperthermia, this hyperthermia is not easily reversed and is

associated with a genetic predisposition to stress, which results in aberrant metabolism. Additionally, these pigs are genetically predisposed to developing malignant hyperthermia (Mitchell & Heffron 1980; 1982). Capture-induced hyperthermia, on the other hand, can be reversed easily, and it appears not to be the result of malignant hyperthermia, as it occurs when animals are captured with or without exercise, and with or without drugs, and the drugs that are used have not been shown to cause malignant hyperthermia (Chapter 2 and 3). Furthermore, malignant hyperthermia has been shown not to be the cause of capture myopathy (Antognini et al. 1996). I also have shown that capture-drugs, with differing effects on thermoregulation, do not influence the magnitude of capture-induced hyperthermia (Chapter 3). Therefore, the pharmacological properties of capture-drugs also are not the primary cause of capture-induced hyperthermia. However, these drugs may cause thermal lability during chemical immobilization and therefore may play an important role in the changes in body temperature post the initial capture and after removal of the initial stressor. Therefore, in environments with high heat loads, drug-induced thermal lability may compound capture-induced hyperthermia and all efforts should be made to reduce heat gain in immobilized animals, thereby reducing the complications associated with capture-induced hyperthermia.

I believe that the major factor contributing to the magnitude of capture-induced hyperthermia is stress. I have shown that individual animals react differently to capture and that their differing stress levels can be correlated to the magnitude of the hyperthermia that they develop (Chapter 2 and 3). I have also shown that if animals are habituated to handling, capture and boma housing, their stress response and capture-induced hyperthermia becomes blunted (Chapter 2). In addition, stress

appears to be the only possible explanation for the precipitous rises in body temperature seen in animals captured in confinement where activity levels and environmental heat loads were low (chapter 2). Also, the stress of handling conscious net-captured animals causes a larger hyperthermia than that caused by high intensity exercise before net-capture (chapter 2). Furthermore, although not describe in the publications of this thesis, I have noticed similar hyperthermias in impala which were disturbed by the presence of humans but were not chased or captured; similar disturbance-induced hyperthermias have been documented in wild penguins (Regel & Putz 1997). Therefore, I concluded that the primary cause of capture-induced hyperthermia is stress, and I propose that the magnitude of this stress-induced hyperthermia exceeds that of exercise-induced and probably all other hyperthermias that may occur during capture.

Although stress is the predominant cause of capture-induced hyperthermia, it is still unclear what the mechanisms are that cause the increase in heat production in stress-induced hyperthermia. Therefore, until such time that these mechanisms are understood, and alternative approaches can be proposed, the best method of reducing capture-induced hyperthermia is to reduce stressful stimuli and the perception of stress during capture procedures.

6.2 Opioid-induced respiratory depression and its reversal

Opioid-induced respiratory depression is a common adverse event that occurs when wild animals are immobilized chemically (McKenzie 1993; Meltzer & Kock 2006b). The primary signs of opioid-induced respiratory depression are believed widely to be brought about by the depressive effects of opioids on respiratory neuronal function, which results in a depression in rhythmogenesis and pulmonary ventilation (McCrimmon & Alheid 2003; Pattinson 2008; Santiago & Edelman 1985), in turn causing hypoxia and hypercapnea (Lumb 2005). In my chemically immobilized goats and impala, I found that the opioid etorphine caused hypoxia and hypercapnea (Chapter 4 and 5). However, the partial pressures of oxygen were not indicative of a pure hypoventilation, but also were related to a decrease in transmembranal oxygen exchange, as revealed by an increase in the gradient of the partial pressures of oxygen between the alveoli and arterial blood (A-a gradient). I assumed that the decrease in oxygen exchange was brought about by a ventilation perfusion mismatching, caused predominantly by a decrease in pulmonary perfusion. Although I did not measure pulmonary blood flows or pressures, my assumptions were based on the findings that etorphine causes pulmonary vasoconstriction (Heard et al. 1996) and chemical immobilization causes systemic sympathetic activation (Meltzer & Kock 2006b; Roquebert & Delgoulet 1988).

As expected, most of the serotonergic ligands that I tested in goats and impala improved opioid-induced depression of respiratory rhythmogenesis, a finding similar to that in rats (Manzke et al. 2003; Richter et al. 2003; Sahibzada et al. 2000). This improvement in rhythmogenesis increased respiratory rate, but it did not necessarily improve ventilation. In goats (Chapter 4) I found that the distinct rhythmogenic

effects of the 5-HT_{1A} and 5-HT_7 agonists 8-OH-DPAT had no effect on ventilation, as the $PaCO_2$ remained elevated, but it did improve the PaO_2 through improving oxygen exchange. Initially I speculated that the improvement of oxygen exchange was brought about by 8-OH-DPAT's actions on 5-HT_7 receptors in the pulmonary vasculature, which would have caused vasodilatation (Morecroft & MacLean 1998). However, in impala (Chapter 5), when I tested buspirone, which does not activate 5-HT_7 receptors, but is an agonist at 5-HT_{1A} receptors, oxygen exchange also was improved. In addition, I found that pimozide (Chapter 5), an agonist of 5-HT_7 receptors, did not improve oxygen exchange or the PaO_2, and nor did it improve rhythmogenesis as previously suggested for rats treated with 8-OH-DPAT (Richter et al. 2003). Therefore I conclude that the beneficial effects of the 5-HT_{1A} agonists 8-OH-DPAT and buspirone during chemical immobilization are not through their rhythmogenic effects but rather through their effects on pulmonary blood flow, which I believe is brought about by their ability to reduce sympathetic tone (Blessing 2004; Dabiré et al. 1990; Ootsuka & Blessing 2003; Saxena & Villalón 1990; Zwieten 1996).

The 5-HT_4 agonist zacopride did improve ventilation; it decreased the $PaCO_2$ in chemically immobilized goats (Chapter 4). It also improved oxygen exchange, an effect which most likely can be attributed to 5-HT_4-induced venodilation in the pulmonary vasculature (Cocks & Arnold 1992). In impala (Chapter 5), the 5-HT_4 agonist metoclopramide also improved oxygen exchange and the PaO_2, but it did not improve ventilation. Therefore the beneficial effects of 5-HT_4 agonists are similar to those of the 5-HT_{1A} agonists in that they improve oxygen exchange, but depending on the agonist, or the species it is used in, they also may have the added advantage of improving ventilation.

Importantly, neither the 5-HT_4 or 5-HT_{1A} agonists reversed the immobilizing effects of the opioid etorphine. Furthermore, as shown in chapter 4, if they are combined with the opioid, they have the added benefit of reducing the time that the animals take to become recumbent, an effect that could be attributed to serotonin's role in catatonia, catalepsy and motility (Broekkamp et al. 1988; Costall & Naylor 1975). The decrease in time to recumbency is not only important in making capture operations more efficient, but is vital, as shown in chapter 3, in reducing the animal's exposure to a stressor, and subsequently reducing the magnitude of the capture-induced hyperthermia, thus further reducing metabolism and further oxygen demand.

6.3 Recommendations and conclusions

Although stress-induced hyperthermia is a well documented occurrence, little is known about the mechanisms involved in its generation (Oka et al. 2001; Olivier et al. 2003; Vinkers et al. 2008). Because stress is a major factor in capture-induced hyperthermia, I believe that stress-induced hyperthermia is a major component of capture-induced hyperthermia. Therefore, gaining a better understanding of the mechanisms involved in stress-induced hyperthermia may help future attempts at limiting capture-induced hyperthermia.

At present, what is known about stress-induced hyperthermia is that anxiolytic drugs reduce or abolish its occurrence in mice (Bouwknecht et al. 2007; Olivier et al. 2003; Vinkers et al. 2008). Anxiolytics which have 5-HT_{1A} agonist effects primarily have been shown to antagonise stress-induced hyperthermia in rodents (Lecci et al. 1990; Ootsuka & Blessing 2003). These anxiolytics may be important agents to include in a

cocktail of drugs used to dart an animal as they may reduce capture-induced hyperthermia and at the same time improve blood oxygen levels. Other anxiolytics that antagonise stress-induced hyperthermia, and which have not been tested for this purpose in ungulates, are the benzodiazepines and α_1-adrenoreceptor antagonists (Bouwknecht et al. 2007; Oka et al. 2001; Olivier et al. 2003). It is also worth noting that α_2-adrenoreceptor agonists and D_2-antagonists, which are classes of drugs commonly used as sedatives and tranquilizers during capture, do not antagonise stress-induced hyperthermia (Bouwknecht et al. 2007; Olivier et al. 2003). Similarly, opioids and their antagonists also are not effective, and neither are anti-pyretics (Bouwknecht et al. 2007; Olivier et al. 2003; Vinkers et al. 2009; Vinkers et al. 2008).

In addition to little being known about the mechanisms involved in the generation of stress-induced hyperthermia, little is known about the effects of capture-induced hyperthermia on an animal. Currently most deaths post-capture are attributed to capture myopathy and although hyperthermia has been implicated as contributing to the pathology of capture myopathy (Antognini et al. 1996; Cheney & Hattingh 1987; Gericke et al. 1978; Meltzer & Kock 2006b), its role needs to be elucidated. If deaths post-capture are attributed, by default, to capture myopathy, other pathologies related to hyperthermia may be missed and the importance of treating or preventing hyperthermia may be overlooked. For example, hyperthermia may induce other lethal pathologies like endotoxic shock and a systemic inflammatory response which may lead to multiple organ dysfunction (Bouchama & Knochel 2002; Leon 2006; Yan et al. 2006). Furthermore, anorexia and the inability to adapt to new environments post-capture, which is a major concern in some species, especially white rhino in bomas (McKenzie 1993), may have its origins in the capture process where hyperthermia

may cause gastrointestinal damage with subsequent endotoxaemia and cytokine release (Leon 2006; 2007). This process is known to cause a post-hyperthermic fever (Leon 2006; 2007) and sickness behaviour which will result in anorexia and depression (Maier & Watkins 1999). Therefore, future studies should investigate the effects of hyperthermia during and after capture, and effective treatments of hyperthermia should be established.

Future studies should also investigate the treatment of the other systemic adverse effects of hyperthermia and stress. A consequence that I have not investigated in my thesis, but which also is of utmost importance, is the acidotic effect of hypoventilation and stress-induced and hyperthermia-induced anaerobic metabolism. Acidaemia has been implicated in causing per-acute deaths during capture, as it causes hyperkalaemia and myocardial dysfunction (Harthoorn 1976). Acidaemia has also been implicated in causing pulmonary hypertension (Harthoorn & Young 1976). Although bicarbonate treatment normalises potassium concentrations (Gericke & Belonje 1975), decreases pulmonary hypertension (Harthoorn & Young 1976), and reduces mortality (Harthoorn et al. 1974; Harthoorn & Young 1976), currently it is not often used post-capture. Therefore future studies should also readdress the treatment of acidaemia.

An increasingly common practice in the field is to administer or insufflate oxygen to chemically immobilized animals that exhibit opioid-induced respiratory depression (Bush et al. 2004; Kock et al. 2006). Essentially this practice corrects hypoxia, but it also may be helpful at reducing anaerobic metabolism and metabolic acidosis in hyperthermic and stressed animals. Although oxygen supplementation and

insufflation may reduce metabolic acidosis, it will not correct respiratory acidosis, but it may compound it by reducing hypoxic respiratory drive and ventilation (Paterson et al. 2009). Another disadvantage is that it can be applied to an animal only once an animal is fully immobilized and an oxygen cylinder is available at the animal's side. Oxygen cylinders are cumbersome in the field, especially if more than one cylinder is needed. Therefore, although it is helpful, it is not always practical to administer or insufflate oxygen to animals with hyperthermia or respiratory depression. Additionally, I have found that respiratory depression is most severe directly after an animal becomes recumbent, at a time when humans, and oxygen cylinders, are not yet at an animal's side. Therefore, it would be ideal to have a drug that could be incorporated in a dart that would reduce capture-induced hyperthermia and prevent respiratory depression. I believe that serotonergic ligands have the potential to fulfil both these requirements. Serotonin not only plays a major role in the control of body temperature (Bligh 1979; Cremer & Bligh 1969), but as I have already mentioned, some of the anxiolytic serotonergics have the ability to antagonise stress-induced hyperthermia. Furthermore, serotonergic ligands also appear to enhance the ability of the opioid drugs to immobilize the animal rapidly, i.e. shorten the "knock down" time.

Contrary to what previous investigators may have assumed, as I have found, the positive rhythmogenic effects of the serotonergic ligands do not necessarily translate into an improvement of ventilation. My research did not reveal why not. It may be that the ligands that I used were not all that specific for the target receptor or may not have been potent enough. It could also be that the positioning of the animal, and the catatonic effects of the opioids, limited the depth of breathing. However, what is clear is that the ligands that I used did have an important effect on pulmonary performance

and they did improve blood oxygenation by improving transmembrane oxygen exchange. Improvement of oxygen exchange, presumably by improving pulmonary perfusion, may be the most important way of correcting blood oxygen pressures during chemical immobilization, and any other procedure where opioids are used. The serotonergic ligands that I used did not entirely restore the A-a gradients, so it appears that there is further scope to improve pulmonary perfusion and oxygen exchange. However, it is essential to first establish what the mechanisms are that cause this reduction in pulmonary perfusion. Currently we know that immobilizing opioids cause pulmonary hypertension (Heard et al. 1996) but how they have this effect is not known. In some species, the effect is mediated by opioid-induced release of histamine (Hakim et al. 1992), but this effect does not occur in all species (Lumb 2005). Another important cause of pulmonary hypertension, especially during capture, could be related to the activation of the sympathetic nervous system (Harthoorn & Young 1976; Heard et al. 1996). Opioids too may have a direct effect on the pulmonary vasculature, or may cause indirect vasoconstriction by causing localized hypoxic-induced pulmonary vasoconstriction. Once the mechanism is established, future investigations should determine whether blood oxygenation can be further enhanced by further improving pulmonary perfusion. Furthermore, it is also important to determine whether agents that improve ventilation and oxygen exchange also improve tissue perfusion and tissue oxygenation. These effects may be particularly important in muscle, the organ primarily affected during capture (Harthoorn 1976).

To conclude, I have shown that capture-induced hyperthermia is caused predominantly by stress rather than by the effects of physical activity, environmental conditions, or the pharmacological effects of the capture drugs. I suggest that limiting

the magnitude and duration of this hyperthermia requires minimising the time an animal is exposed to a stressor, i.e., the time from first encounter until the animal becomes sedated and chemically immobile or confined. If animals are not immobilized but are confined in a foreign environment, steps should be taken to reduce further exposure to stressors. If animals are chased before capture, steps also should be taken to limit additional heat production by decreasing the length, duration and especially the intensity of pre-capture exercise. Restricting capture to cool times of the day, or cool months of the year, will not protect animals from developing capture-induced hyperthermia; contrary to the folk lore amongst capture teams. To reduce capture-induced hyperthermia, and its associated morbidity and mortality, capture techniques that invoke lower stress responses should be used. Additionally, the pharmacology of the drugs that are used to capture animals, if they do not have anxiolytic effects, will not directly influence capture-induced hyperthermia, but drug cocktails should be selected so as to restrict time and distances to recumbency, thereby limiting the stress and metabolic heat production. However, in some species the drugs with the best knock down times have the greatest respiratory depressant effects, and therefore caution should be exercised when selecting drugs based purely on their immobilizing properties.

Therefore, drug cocktails also should be selected following consideration of their respiratory depressant effects, and once an animal is immobilized it should be monitored to track whether it develops hypoxia. If it does develop hypoxia appropriate steps should be taken to reduce this hypoxia. One of these steps would be to administer serotonergic ligands which have agonist effects at 5-HT_4 and 5-HT_{1A} receptors. Although no serotonergic ligands are registered for use in wildlife, the two

serotonergic drugs buspirone and metoclopramide, which are available readily for clinical use in man and other mammals, could be used off/extra-label. These drugs, and the other serotonergic ligands that we tested, have the ability to improve respiratory rhythmogenesis but their main beneficial effect was not that of improving ventilation but rather of improving oxygen exchange, presumably by improving pulmonary perfusion and ventilation perfusion ratios. I believe that the effectiveness of these drugs at reversing opioid-induced respiratory depression during chemical immobilization will be fully realised when the drugs are included in a dart, so that their rhythmogenic effects and their effects on pulmonary perfusion will occur right from the onset of chemical immobilization. Additionally, I have shown that serotonergic ligands can enhance catatonic immobilization and therefore their use in a dart may also improve “knock down” times, thereby reducing stress and capture-induced hyperthermia. However, future studies will need to investigate this action further.

Although I have shown that serotonergic ligands can partially improve opioid-induced hypoxia, they did not eliminate it, so there exists a large scope to further improve respiratory function and blood oxygenation in animals under opioid immobilization. I believe that efforts to do so should focus not only on improving ventilation but also on further improving pulmonary perfusion and ventilation-perfusion ratios. These effects and other efforts to reduce capture-induced hyperthermia hopefully will reduce the morbidity and mortality associated with capture.

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