RESEARCH PAPER

Dose-effect study of the serotonin agonist R-8-OH-DPAT on opioid-induced respiratory depression in blesbok (*Damaliscus pygargus philipsi*) and impala (*Aepyceros melampus*)

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Abstract

Objective To determine whether the R-enantiomer of 8-hydroxy-2-(di-n-propylamino) tetralin (R-8-OH-DPAT) alleviates respiratory depression in antelope species immobilized with etorphine. The experiment also aimed to establish the most clinically effective dose of this serotonin 5- HT_{1A} receptor agonist.

Animals A group of six female blesbok and six female impala.

Study design Each animal was subjected to four immobilization treatments in a prospective four-way crossover design—control treatment consisting of only etorphine at 0.09 mg kg^{-1} and three treatments consisting of etorphine at 0.09 mg kg^{-1} combined with 0.005, 0.02 and 0.07 mg kg^{-1} of R-8-OH-DPAT, respectively. Induction, quality of immobilization and recovery were monitored in each treatment. Physiological variables including heart rate, respiratory rate, arterial blood pressure and blood gases were measured for 35 minutes during immobilization. A linear mixed model was used to assess the effects of treatments over the recumbency period.

Results R-8-OH-DPAT did not influence induction, immobilization or recovery scores. Respiratory rate in blesbok was increased in the medium- and high-dosage R-8-OH-DPAT treatment group. However, this increased respiratory rate did not translate into improvements of arterial partial pressure of oxygen (PaO_2) values in the blesbok. The medium and higher dosages of R-8-OH-DPAT in impala led to an improved PaO_2 as well as to decreased opioid-induced tachycardia during the first 10 minutes of immobilization.

Conclusions and clinical relevance Previous reports indicated that the racemic mixture of 8-OH-DPAT injected intravenously had a positive effect on blood-gas values in etorphine-treated hypoxemic goats. In this experiment, similar effects could be seen in impala at the higher dosage rates of R-8-OH-DPAT. However, failure to achieve an improvement of blood-gas values in blesbok was an unexpected result. It could be speculated that the dosage, species-specific differences of serotonin receptors or the use of the R-enantiomer of 8-OH-DPAT might play a role.

Keywords 8-OH-DPAT, etorphine, respiratory depression, wildlife immobilization.

Introduction

Sales of potent opioids in 2016 suggest that over 150,000 wild animals, mostly herbivores, were chemically immobilized in South Africa for various clinical, management or research purposes (Wildlife Pharmaceuticals SA 15/1/2018, unpublished). Chemical immobilization is the only way to safely capture and treat or relocate most wildlife species, and veterinarians have a responsibility to use the safest combinations of chemicals to prevent adverse events like injury or death of the animals.

Immobilizing wild animals under field conditions is associated with significant challenges. The age, health and pregnancy status is often unknown and the animals are sometimes chased over long distances before the dart can be administered. Feed and water intake prior to darting is usually uncontrolled and immediate approach after the immobilizing drugs take effect is often not possible because of terrain constraints and the distance the animals run after darting. At times, an animal may be unattended for up to 30 minutes after darting before it can be safely repositioned and basic anaesthetic monitoring can commence. It is only from this time that actions to combat negative side effects of the immobilization can be taken.

Potent opioids such as etorphine or thiafentanil are often used for the immobilization of wild herbivores (Haigh 1990; Kock & Burroughs 2012). One disadvantage of using these potent opioids is that they often cause clinically significant respiratory depression, which is mostly associated with their mu-opioid receptor activity (Haigh 1990; Kock & Burroughs 2012). Activation of mu-opioid receptors in the respiratory centres of animals depresses neurons that generate the normal respiratory rhythm. At the same time, activation of mu-opioid receptors on chemoreceptors in the brain stem, on the aortic arch and the carotid bodies depresses the normal respiratory drive as these chemoreceptors become less sensitive to activation by hypercapnia, hypoxemia and acidemia. This decreased sensitivity in turn leads to a reduction of the respiratory frequency and tidal volume (Buss & Meltzer 2001; McCrimmon & Alheid 2003). Furthermore, it has been suggested that pulmonary vasoconstriction, caused by the sympathomimetic actions of etorphine, decreases pulmonary perfusion. This effect leads to impaired diffusion of oxygen through the alveolar membrane (Meyer et al. 2015). Studies have found that serotonergic ligands, specifically the racemic form of 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), reverse respiratory depression through their effects on the brainstem respiratory neurons (Lalley et al. 1994; Sahibzada et al. 2000). In addition, 8-OH-DPAT improved alveolar oxygen diffusion in goats without affecting catatonia and sedation caused by opioids (Meyer et al. 2006). The R-enantiomer of 8-OH-DPAT (R-8-OH-DPAT), compared with the racemic form, has a higher specificity at the 5-HT_{1A} receptors and thus possibly greater effects. It is postulated that because of this, the R-enantiomer may produce a more effective reversal of opioid-induced respiratory depression (Hadrava et al. 1996; Yu et al. 1996; Lejeune et al. 1997; Christoffersen & Meltzer 1998).

Impala (*Aepyceros melampus*) and blesbok (*Damaliscus pygargus philipsi*) were chosen for this experiment as they are abundant and readily available in the study area. They are also two species commonly immobilized with potent opioids. This study aimed to determine the ability of R-8-OH-DPAT to prevent opioid-induced respiratory depression in blesbok and impala when administered in combination with etorphine in a dart. The experiment also aimed to establish whether effects of R-8-OH-DPAT might be species-specific and to determine the most clinically effective dose of R-8-OH-DPAT. It was hypothesized that R-8-OH-DPAT would mitigate opioid-induced respiratory depression in both wild ungulate species without affecting the quality of immobilization.

Materials and methods

The study was approved by the Murdoch University Animal Ethics committee (R2923/17) and by the Wildlife Pharmaceuticals Animal Ethics Committee (WPAEC—DPATBLES—11-B).

A group of six female impala (34.5 ± 4.6 kg) and six female blesbok (56.6 ± 6.5 kg) were used for this study and held at the Wildlife Pharmaceuticals research facility, South Africa (25°31′5.2″ S; 31°06′50.8″ E). Research animals were chosen to be of same sex, similar size and in good health to reduce confounding variables. Males were excluded from this study for practical (housing) reasons.

The enclosures consisted of several compartments that were approximately 6 × 8 m in size and were constructed from sturdy gum tree poles. The impala and blesbok were held separately in groups of six animals per enclosure.

Animals were wild captured. After an initial adjustment period of 2 weeks after delivery, the animals were immobilized, marked and subjected to a veterinary health examination which included a full blood cell count, a blood smear and a liver and kidney function test. At this occasion, the animals under immobilization were weighed while lying in sternal position in a stretcher and lifted with a hand-held precision scale, which was attached to a pole (Anyload OCSL Mini Crane Scale; Anyload Transducer Co. Ltd., Canada).

The immobilizing drug etorphine as well as R-8-OH-DPAT were administered intramuscularly (IM) via remote injection by darting. Each animal was darted by means of a carbon dioxide (CO_2) gas-powered dart projector (X-Caliber; Pneu-Dart. Inc., PA, USA). The darts used were 1 mL (impala) and 1.5 mL (blesbok) P-type Pneudarts with 1.9 cm barbed needles (Pneu-Dart. Inc., PA, USA). Animals were darted on four separate occasions with a wash-out period of 2 weeks between each occasion. A total of six animals were processed on each trial day. Each animal was weighed again during the second occasion and drug dosages adjusted according to weight for the following occasions. The drug dosage of etorphine (Captivon, 9.8 mg mL^{-1}; Wildlife Pharmaceuticals SA (Pty) Ltd, South Africa) was 0.09 mg kg^{-1} in all animals. Etorphine was combined with various dosages of R-8-OH-DPAT hydrobromide (Tocris Bioscience, Bristol, UK, catalogue number 1080). R-8-OH-DPAT treatments were: 1) control dose consisting of etorphine only, 2) low dose at 0.005 mg kg^{-1}, 3) medium dose at 0.02 mg kg^{-} and 4) high dose at 0.07 mg kg^{-1}. The darts

were filled to capacity with sterile water. R-8-OH-DPAT was dissolved in a pharmaceutical laboratory under aseptic conditions in water for injection to 1 mg mL^{-1} and 5 mg mL^{-1} within 24 hours before each trial. Stability of the chemical in solution was confirmed during a previous bioavailability experiment (Pfitzer et al. 2019). The highest dosage of 0.07 mg kg^{-1} was chosen based on literature on goats according to which 0.1 and 0.5 mg kg^{-1} of the racemic form were injected intravenously (IV) (Herman et al. 2001; Meyer et al. 2006). Dosages were also determined based on the results of a bioavailability study in goats (Pfitzer et al. 2019). During the latter study, the IV administration of 0.1 mg kg^{-1} R-8-OH-DPAT led to serotonergic side effects in goats (Pfitzer et al. 2019). Consequently, the R-8-OH-DPAT dosages for this wildlife study were chosen to be lower than 0.1 mg kg^{-1}. The lowest dosage of 0.005 mg kg^{-1} was chosen based on literature on rats where effects on respiratory depression of racemic 8-OH-DPAT were found from 0.01 mg kg^{-1} (Sahibzada et al. 2000). Due to its higher specificity at the 5-HT_{1A} receptors, R-8-OH-DPAT was predicted to be more potent than the racemic form (Hadrava et al. 1996; Yu et al. 1996; Lejeune et al. 1997). Therefore, the lowest dosage was chosen at 0.005 mg kg^{-1} and the medium dosage double that of the first effective dosage reported in rats, namely 0.02 mg kg^{-1}.

The trial was performed as a four-way crossover dose-response study on four trial occasions. Each animal was treated once on each trial occasion. On the first trial date, animals were allocated to a treatment dose schedule by means of a random-number generator (Microsoft Excel for Mac version 16.26; Microsoft Corporation, WA, USA). All treatments were allocated at least once on each trial date. Care was taken that treatments were spread out over various times of the day. The sample size of six animals was small but considered to be sufficient. As it is logistically difficult to hold large numbers of wild animals, studies of this kind are often conducted using small number of animals (Huber et al. 2001; Howard et al. 2004; Risling et al. 2011; Sawicka et al. 2015).

As soon as an animal became immobile and could be approached, it was blind-folded and cotton wool inserted into the ears to minimize external stimuli. The animal was then loaded onto a stretcher and carried out of the enclosure to an experimental monitoring table where it was placed in sternal position with the head elevated.

Recording of physiological variables began at 5 minutes after an animal became recumbent. Physiological variables were recorded every 5 minutes until 35 minutes after recumbence. Rectal temperature (RT) was measured using a modified hand-held digital thermometer (Hanna Checktemp 1; Hanna Instruments (Pty) Ltd., NE, USA). The environmental temperature and barometric pressure were recorded during each immobilization. Barometric pressure was measured by the EPOC portable blood-gas analyser (EPOC Blood Analysis System; Epocal, ON, Canada) and the environmental temperature was measured by the Weather^{+} Bluetooth Sensor (Oregon Scientific, OR, USA). Respiratory rate (f_R) was measured by visual observation of chest expansions as well as by auscultation with a stethoscope for 1 minute (Littman Classic II SE; Littman, MN, USA). The auricular (*Arteria auricularis*) or pedal artery (*A. digitalis*) was catheterized using a 21 gauge catheter (Jelco IV catheter radiopaque; Smith Medical International, UK) to measure intra-arterial blood pressure using the Deltran II pressure transducer (Utah Medical, UT, USA) connected to an IntraTorr blood pressure monitor (IntraTorr; IntraVitals, UK). This device also measured heart rate (HR). Arterial blood was also drawn from these catheters for blood-gas analysis. Arterial blood samples were collected anaerobically in a heparinized blood-gas syringe (BD A-Line; Becton Dickinson & Co, UK) at 5, 10, 15, 20 and 30 minutes after recumbence. Arterial blood was analysed by a portable blood-gas analyser using EPOC BGEM test cards (BGEM smart cards; Epocal, ON, Canada) to determine blood gases within 5 minutes after sampling. Measurements on the test cards included blood gases as well as lactate, glucose, creatinine, sodium, potassium, calcium and haematocrit. The alveolar-arterial oxygen (A-a) gradient corrected to RT was calculated as described by Meyer et al. (2010).

All measurements were made outdoors in a shaded area from 08:00 to 12:00 and from 14:00 to 17:00, respectively, to avoid hot temperatures at midday.

The immobilization of all animals was reversed with naltrexone [Trexonil 50 mg mL^{-1}; Wildlife Pharmaceuticals SA (Pty) Ltd, South Africa] injected IV at a ratio of 20 mg naltrexone to 1 mg etorphine as soon as the 35 minute monitoring period was over. Induction and recovery were monitored, and each animal was subjected to an induction score (referring to speed and quality), immobilization score (referring to motor responses during the monitoring period) and recovery score (referring to speed and smoothness of recovery after the IV administration of naltrexone; Table 1).

Data analysis

Analyses were performed with Genstat Version 19 (VSN International Ltd., UK) to determine whether there was an effect of the various R-8-OH-DPAT dose rates on the physiological variables of interest. A linear mixed model was fitted to the data. Since the R-8-OH-DPAT dose rate effect could be expected to show a linear trend, the R-8-OH-DPAT dose rate effect was subdivided into a linear trend and a nonlinear trend. The effect of time after recumbence was not expected to be linear, so a random slope model was not considered. The fixed model included a linear effect of R-8-OH-DPAT, a nonlinear effect of R-8-OH-DPAT, the effect of time after recumbence and

Table 1 Induction, immobilization and recovery scores

Score	1	2	3	4	5
Induction	Slight ataxia, animal goes into recumbence within 5 minutes and does not get up again	Ataxia, animal might go down twice before final recumbence	Longer ataxic phase (15 minutes). Repeated recumbence before approach is possible.	Animal is ataxic but does not go down and needs to be physically captured or darted again	No signs of drug taking effect
Immobilization	Animal attempts to stand and needs to be re-dosed, risk to handlers	Tongue movement, chewing, spontaneous motor activity, struggling during manipulation (sedated)	Tongue is relaxed, but reactive to touch and thus intubation not possible, muscular rigidity, can be handled safely (light anaesthesia)	Smooth, complete relaxation, extractable tongue, loss of pedal reflex, no involuntary tail movements, (moderate to deep anaesthesia)	Too deep, no reflexes, severe cardiorespiratory depression
Recovery	Smooth and rapid transition from assisted sternal recumbence to unassisted sternal recumbence. Animal stands in one attempt and is sufficiently recovered to walk with only slight ataxia within 3 minutes after administration of drug antagonists (excellent)	Some imbalance in unassisted sternal recumbence. Animal displays moderate ataxic movements and may take one or two attempts to get up. Walks with moderate ataxia and lack of coordination. Recovery to walking occurs within 15 minutes following administration of drug antagonists (good)	Animal needs assistance to stay in sternal recumbence. Makes numerous attempts to stand but frequently falls before being successful and displays marked ataxia when walking. Recovery to walking in excess of 15 minutes following administration of drug antagonists (fair)	Animal needs to be assisted in sternal recumbence for more than 15 minutes following the administration of drug antagonists, is not responsive to stimuli and makes no attempt to stand up for over 30 minutes (poor)	Animal does not recover and dies or has to be euthanized

interactions between treatment and time effects. The random model included terms for animal, trial date, animal by trial date and animal by trial date by minutes after recumbence. The residual variance/covariance model included correlations between measurements made on the same animal on the same trial date and different variances for each animal. Nonsignificant random effects and co-variances were removed from the model before the significance of fixed effects was assessed. A p value of <0.05 was considered significant.

For evaluating f_R, the data was transformed logarithmically prior to analysis. This transformation was required due to the increase in residual variance as f_R increased more than 10-fold.

HR in beats minute^{-1}, f_R in breaths minute^{-1}, systolic (SAP), diastolic (DAP) and mean arterial pressure (MAP), arterial blood pH, arterial partial pressure of carbon dioxide ($PaCO_2$) corrected to RT, arterial partial pressure of oxygen (PaO_2) corrected to RT, A-a gradient corrected to RT were statistically evaluated.

Standard errors of differences (SEDs) are shown with means so that the reader can assess whether differences between means are statistically significant. They can be used to calculate a t value [least significant difference (LSD), 5%] to compare the difference between means and zero at the 5% level of significance.

Time to final recumbence, induction, immobilization and recovery scores were analysed for normality using the Shapiro–Wilk test. If the data was parametric, repeated measures analysis of variance (ANOVA) was used to compare the repeated measurements in each R-8-OH-DPAT anaesthetic regime (0, 0.005, 0.02 and 0.07 mg kg^{-1}). If the data was nonparametric, the Friedman's ANOVA test was used. Values of $p > 0.05$ were considered statistically significant.

Data was summarized and presented as median and interquartile range. Statistical analysis was performed on IBM SPSS for Windows, Version 24 (IBM Corp., NY, USA).

Results

Environmental temperatures were 23.3 ± 5.9 °C and 23.8 ± 4.2 °C during the blesbok and impala experiments, respectively.

The animals' RTs varied but were all within physiologically acceptable limits (Table 2).

Significant treatment effect and treatment and time interactions could be identified in some of the physiological variables. In both species, the majority of variables were affected by time (Table 3). A summary of measured physiological variables of the treatments for both species with SED is given in Table 2.

There were no statistically significant differences between treatment group medians for time to recumbence and induction, immobilization and recovery scores in blesbok or impala as determined by Friedman's ANOVA (Table 4).

Table 2 Physiological values (means) for 5, 10, 20 and 30 minutes after recumbence in blesbok and impala immobilized with 0.09 mg kg^{-1} etorphine and three dosages of R-8-OH-DPAT, namely low (0.005), medium (0.02) and high (0.07 mg kg^{-1}). Physiological values evaluated were heart rate (HR), respiratory rate (f_R), systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP) arterial partial pressure of carbon dioxide ($PaCO_2$), arterial partial pressure of oxygen (PaO_2), alveolar-arterial oxygen gradient (A-a gradient), arterial blood pH, rectal temperature (RT), lactate and applicable standard error of differences (SED)

Variable	Treatment	Time after recumbence of impala			
		5 minutes	10 minutes	20 minutes	30 minutes
HR (beats $minute^{-1}$)	Control	54	46	46	44
	Low	45	44	41	38
	Medium	54	49	45	41
	High	52	49	47	41
SED within treatment		2.9	SED within time		*3.9*
log_e f_R (breaths $minute^{-1}$)	Control	2.7 (14)	2.7 (15)	2.4 (11)	2.3 (10)
	Low	3.2 (24)	2.8 (16)	2.7 (14)	2.4 (11)
	Medium	3.8 (45)	3.6 (37)	3.1 (23)	3.0 (21)
	High	3.2 (24)	3.0 (21)	2.9 (19)	2.9 (19)
SED within treatment[a]		0.13	SED within time[a]		0.27
SAP (mmHg)	Control	151	152	142	137
	Low	144	148	140	139
	Medium	161	158	152	151
	High	147	152	147	148
SED within treatment		3.0	SED within time		7.6
DAP (mmHg)	Control	118	118	109	107
	Low	116	115	104	101
	Medium	115	119	118	112
	High	113	120	114	111
SED within treatment		2.3	SED within time		7.3
MAP (mmHg)	Control	134	128	122	121
	Low	127	129	125	118
	Medium	137	135	130	129
	High	130	131	126	125
SED within treatment		3.0	SED within time		5.2
$PaCO_2$ (mmHg) [kPa]	Control	42 [5.6]	43 [5.7]	44 [5.9]	46 [6.1]
	Low	42 [5.6]	43 [5.7]	45 [6.0]	45 [6.0]
	Medium	39 [5.2]	40 [5.3]	45 [6.0]	47 [6.3]
	High	40 [5.3]	44 [5.9]	52 [6.9]	53 [7.1]
SED within treatment		1.5 [0.20]	SED within time		1.8 [0.24]
PaO_2 (mmHg) [kPa]	Control	77 [10.3]	75 [10.0]	74 [9.9]	70 [9.3]
	Low	74 [9.9]	73 [9.7]	71 [9.5]	66 [8.8]
	Medium	78 [10.4]	79 [10.5]	69 [9.2]	66 [8.8]
	High	79 [10.5]	67 [8.9]	65 [8.7]	65 [8.7]
SED within treatment		4.2 [0.56]	SED within time		4.4 [0.59]
A-a gradient (mmHg) [kPa]	Control	17 [2.3]	17 [2.3]	18 [2.4]	19 [2.5]
	Low	21 [2.8]	21 [2.8]	24 [3.2]	20 [2.7]
	Medium	20 [2.7]	17 [2.3]	20 [2.7]	24 [3.2]
	High	16 [2.1]	23 [3.1]	21 [2.8]	18 [2.4]
SED within treatment		2.5 [0.33]	SED within time		2.8 [0.37]
pH	Control	7.44	7.43	7.43	7.42
	Low	7.44	7.44	7.43	7.44
	Medium	7.48	7.46	7.43	7.42
	High	7.49	7.45	7.42	7.40
SED within treatment	0.21	0.013	SED within time		0.014
RT (°C)	Control	38.6	38.9	39.0	39.0
	Low	38.8	39.3	39.2	39.1
	Medium	39.1	39.3	39.4	39.3
	High	38.8	39.2	39.2	39.0
SED within treatment		0.11	SED within time		0.21
Lactate (mmol L^{-1})	Control	2.33	1.79	1.53	1.34

Table 2 (*continued*)

Variable	Treatment	Time after recumbence of impala			
		5 minutes	**10 minutes**	**20 minutes**	**30 minutes**
	Low	1.46	1.20	0.97	0.91
	Medium	1.16	0.99	0.83	0.80
	High	1.45	1.15	1.01	0.86
SED within treatment		0.182	SED with time		0.443
Variable	Treatment	Time after recumbence of impala			
		5 minutes	10 minutes	20 minutes	30 minutes
HR (beats minute^{-1})	Control	157	125	102	93
	Low	137	125	83	72
	Medium	124	116	71	62
	High	103	90	75	64
SED within treatment		10.1	SED within time		13.8
$\log_e f_R$ (breaths minute^{-1})	Control	2.3 (10)	2.0 (7)	1.9 (7)	1.8 (6)
	Low	2.4 (11)	2.2 (9)	2.0 (7)	2.0 (7)
	Medium	2.0 (7)	2.0 (7)	1.7 (6)	1.8 (6)
	High	2.3 (10)	2.2 (9)	1.9 (7)	1.9 (7)
SED within treatment[a]		0.13	SED within time[a]		0.20
SAP (mmHg)	Control	103	103	96	104
	Low	114	117	110	107
	Medium	114	105	103	111
	High	134	118	110	108
SED within treatment		7.2	SED within time		10.1
DAP (mmHg)	Control	83	79	74	77
	Low	89	80	87	76
	Medium	89	77	75	80
	High	102	76	72	76
SED within treatment		5.4	SED within time		8.0
MAP (mmHg)	Control	96	100	83	85
	Low	99	97	91	86
	Medium	97	93	83	91
	High	113	97	86	88
SED within treatment		4.3	SED within time		8.1
PaCO2 (mmHg) [kPa]	Control	61 [8.1]	57 [7.6]	57 [7.6]	55 [7.3]
	Low	61 [8.1]	58 [7.7]	59 [7.9]	58 [7.7]
	Medium	59 [7.9]	58 [7.7]	57 [7.6]	57 [7.6]
	High	59 [7.9]	57 [7.6]	58 [7.7]	56 [7.5]
SED within treatment		1.6 [0.21]	SED within time		2.5 [0.33]
PaO2 (mmHg) [kPa]	Control	48 [6.4]	58 [7.7]	58 [7.7]	60 [8.0]
	Low	45 [6.0]	53 [7.1]	60 [8.0]	63 [8.4]
	Medium	54 [7.2]	57 [7.6]	59 [7.9]	62 [8.3]
	High	53 [7.1]	55 [7.3]	56 [7.5]	61 [8.1]
SED within treatment		3.6 [0.48]	SED within time		4.1 [0.55]
A-a gradient (mmHg) [kPa]	Control	31 [4.1]	21 [2.8]	19 [2.5]	18 [2.4]
	Low	29 [3.9]	18 [2.4]	16 [2.1]	14 [1.9]
	Medium	28 [3.7]	22 [2.9]	19 [2.5]	17 [2.3]
	High	27 [3.6]	23 [3.1]	19 [2.5]	17 [2.3]
SED within treatment		2.5 [0.33]	SED within time		3.0 [0.40]
pH	Control	7.28	7.32	7.35	7.38
	Low	7.31	7.34	7.36	7.37
	Medium	7.30	7.32	7.34	7.36
	High	7.28	7.31	7.35	7.39
SED within treatment		0.008	SED within time		0.016
RT (°C)	Control	39.2	39.6	39.7	39.5
	Low	39.1	39.5	39.4	39.4
	Medium	39.2	39.5	39.3	39.1

(*continued on next page*)

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Table 2 (*continued*)

Variable	Treatment	Time after recumbence of impala			
		5 minutes	10 minutes	20 minutes	30 minutes
	High	39.0	39.2	39.3	38.3
SED within treatment		0.05	SED within time		0.31
Lactate (mmol L^{-1})	Control	5.20	4.46	3.06	1.89
	Low	3.44	2.41	1.25	0.77
	Medium	3.85	3.03	1.98	1.14
	High	4.50	3.57	1.86	1.03
SED within treatment		0.273	SED within time		0.778

[a]SEDs apply only to $\log_e f_R$ means.

Blesbok

There was no significant effect of treatment on the HR of blesbok (Table 3). There was a significant linear treatment effect ($p = 0.003$) as well as a treatment by time interaction ($p = 0.016$) for f_R. The overall mean f_R of the medium dose (26.7 ± 6.5 breaths $minute^{-1}$) and high dose (20.5 ± 5.0 breaths $minute^{-1}$) group was higher than the control (11.5 ± 2.8 breath $minute^{-1}$; Fig. 1). There was no difference between the low-dose treatment and control at any time.

There was no treatment effect on SAP and MAP. However, there was a significant treatment by time interaction for DAP ($p = 0.014$) caused by a larger drop of DAP in the control group and low-dose treatment (Table 3).

There was a significant treatment by time interaction for arterial blood pH ($p = 0.002$). At 5 minutes, the pH values were higher for the high-dose and medium-dose treatment groups (7.49 and 7.48, respectively) than for the low-dose treatment and control groups (7.44 and 7.44, respectively;

Table 3 Significance of treatment effects of R-8-OH-DPAT on certain physiological parameters when used in conjunction with etorphine immobilization in blesbok ($n = 6$) and impala ($n = 6$). Significant results of R-8-OH-DPAT treatment effects, effect of time and interaction of time and treatment ($p < 0.05$) are given in bold. Physiological values evaluated were heart rate (HR), logarithmic values of the respiratory rate ($\text{Log } f_R$), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP), arterial, blood pH (pH), arterial partial pressure of carbon dioxide ($PaCO_2$), arterial partial pressure of oxygen (PaO_2) and the alveolar-arterial oxygen gradient (A-a gradient)

Fixed term	Treatment effect (Combined linear and nonlinear effects of R-8-OH DPAT dosage)	Time effect (minutes after recumbence)	Interaction (treatment time)
Blesbok			
HR	0.308	<0.001	0.635
Log f_R	0.007	<0.001	0.016
SAP	0.420	0.003	0.168
DAP	0.658	<0.001	0.014
MAP	0.197	<0.001	0.739
$PaCO_2$	0.017	<0.001	<0.001
PaO_2	0.065	<0.001	0.385
A-a gradient	0.230	0.218	0.085
pH	0.198	<0.001	0.002
Impala			
HR	0.005	<0.001	0.435
Log f_R	0.422	<0.001	0.952
SAP	0.311	0.028	0.198
DAP	0.998	<0.001	0.032
MAP	0.973	0.007	0.237
$PaCO_2$	0.598	0.001	0.586
PaO_2	0.657	<0.001	0.187
A-a gradient	0.693	<0.001	0.474
pH	0.901	<0.001	0.013

Table 4 Immobilization quality parameters for all six animals of each species. Animals were immobilized with 0.09 mg kg^{-1} etorphine on four occasions as various dosages of R-8-OH-DPAT were added to the mixture. Median and interquartile range (IQR) for all R-8-OH-DPAT treatment groups (0, 0.005, 0.02 and 0.07 mg kg^{-1}) for time to recumbence, mean induction, mean immobilization and mean recovery scores of blesbok ($n = 6$ animals with a total of 24 immobilizations) and impala ($n = 6$ animals with a total of 24 immobilizations). Where scores were not parametrically distributed, difference amongst the treatment groups were analysed using the Friedman's analysis of variance test and p values are quoted

	Time to recumbence in minutes	Induction score	Immobilization score	Recovery score
Blesbok	3.5 (1.3), $p = 0.334$	1 (0)	3 (0.5), $p = 0.656$	1 (0)
Impala	3.6 (1.7), $p = 0.615$	1 (0), $p = 0.875$	4 (1), $p = 0.902$	1 (0)

SED = 0.014). However, this effect was not present after 10 minutes.

There was a significant nonlinear treatment effect ($p = 0.021$) as well as treatment by time interaction ($p < 0.001$) for $PaCO_2$. From 15 minutes, the high-dose treatment lead to significantly more severe hypercapnia than all other treatments. This difference persisted to the end of measurement when $PaCO_2$ of the high-dose treatment measured 53 mmHg (7.1 kPa) and the low-dose treatment measured 45 mmHg [6.0 kPa; SED = 1.8 (0.24)].

There was significant linear treatment effect, irrespective of time on PaO_2 ($p = 0.036$), indicating that hypoxia increased with dose rate. The high-dose treatment led to significantly more pronounced hypoxia [overall mean = 68 mmHg (9.1 kPa)] than when animals were treated with the control, low- and medium-dose treatments [overall mean = 74 (9.9), 71 (9.5) and 73 (9.7) mmHg (kPa), respectively; SED = 1.6 (0.21)].

None of the treatments influenced A-a gradients and mean values for all treatment groups combined ranged from the lowest of 16 mmHg (2.1 kPa) at 5 minutes (high-dose

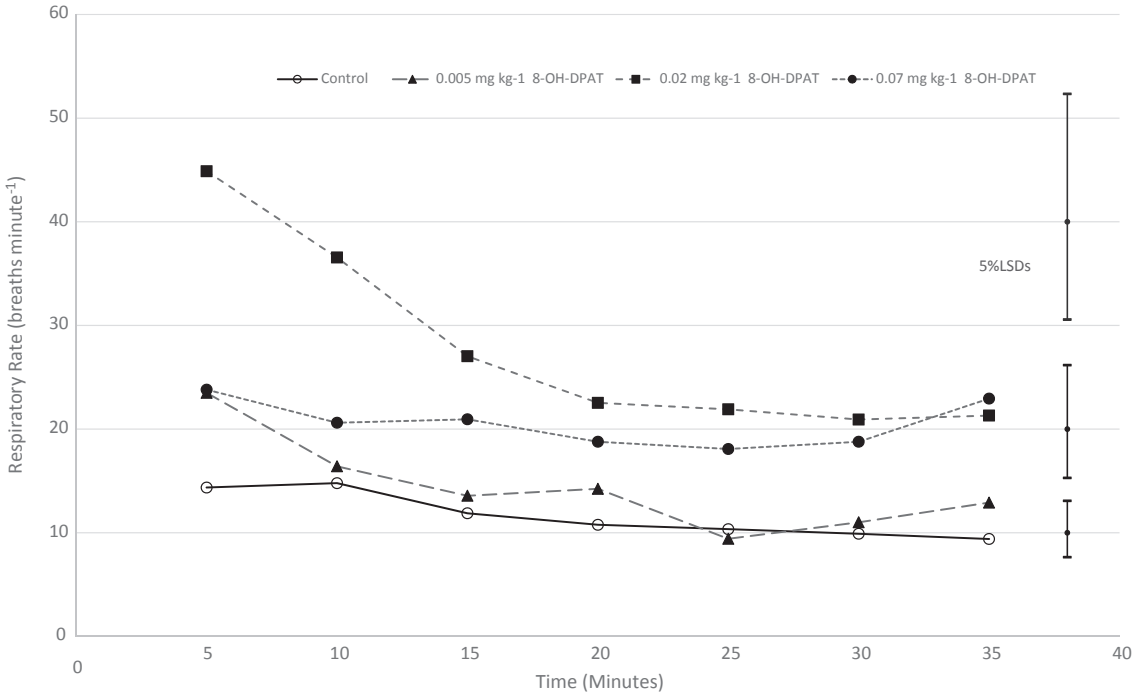

Figure 1 Effect of R-8-OH-DPAT-treatment on the respiratory rate of etorphine-immobilized blesbok ($n = 6$) over time (breaths $minute^{-1}$). Control, low dose (0.005 mg kg^{-1}), medium dose (0.05 mg kg^{-1}), high dose (0.07 mg kg^{-1}). Respiratory rate values are retransformed from logarithms. Error bars are least significant differences (5% LSD) at three levels of respiratory rate (10, 20 and 40). A difference between the means larger than the LSD is considered significant.

treatment) to the highest value of 24 mmHg (3.2 kPa) at 30 minutes [medium-dose treatment; SED = 1.2 (0.16)].

Impala

There was a significant linear treatment effect on the HR of this species ($p < 0.001$), whereby the HR was lower with the higher R-8-OH-DPAT dosages (Table 2). HR declined in a linear manner with all treatments over time.

There was a significant treatment by time interaction for DAP ($p = 0.032$). At 5 minutes, the high-dose treatment led to significantly higher DAP (102 mmHg) than all other treatments, but this difference was not present thereafter (SED = 8.0). MAP and SAP were not affected by the treatments but decreased with time in all treatment groups ($p = 0.007$ and $p = 0.028$, respectively).

At the 5 minute time point, the control and low-dose treatments led to worse hypoxia [48 and 45 mmHg (6.4 and 6.0 kPa)] than the medium- and high-dose treatments [54 and 53 mmHg (7.2 and 7.1 kPa); SED = 3.6 (0.48); Table 3 & Fig. 2]. This difference was not statistically significant and also not detectable at later time points. PaO_2 with all treatments increased significantly over time (Fig. 2).

The A-a gradients determined at RT of all treatment groups declined over time irrespective of the treatment ($p < 0.001$). Mean values for all treatment groups combined ranged from the highest of 31 mmHg (4.1 kPa) at 5 minutes (control treatment) to the lowest mean value of 14 mmHg (1.9 kPa) at 30 minutes [low-dose treatment; SED = 3.0 (0.40)].

Discussion

There were significant cardiorespiratory effects of R-8-OH-DPAT on etorphine-immobilized blesbok and impala. Results of clinical interest that warrant further discussion include the R-8-OH-DPAT-related increase in f_R of blesbok and the R-8-OH-DPAT dose-dependent changes in HR and PaO_2 of impala.

The medium- and high-dose treatment of blesbok in this experiment developed the highest mean f_R, which differed significantly from the control treatment throughout the monitoring period (Fig. 1). This is in accordance with Meyer et al. (2006) who reported that the IV administration of racemic 8-OH-DPAT (0.5 mg kg^{-1}) in goats prevented respiratory depression caused by etorphine. However, this improved respiration in goats did not translate into improved ventilation as the goats were still hypercapnic. Meyer et al. (2006) also reported that despite this, the hypoxia in goats did improve due to an effect of 8-OH-DPAT on the pulmonary circulation, which improved oxygen diffusion.

In contrast to the goats, the medium-dose treatment group of blesbok with the highest f_R did not show any improved PaO_2 values. Mean PaO_2 was slightly lower than the control group.

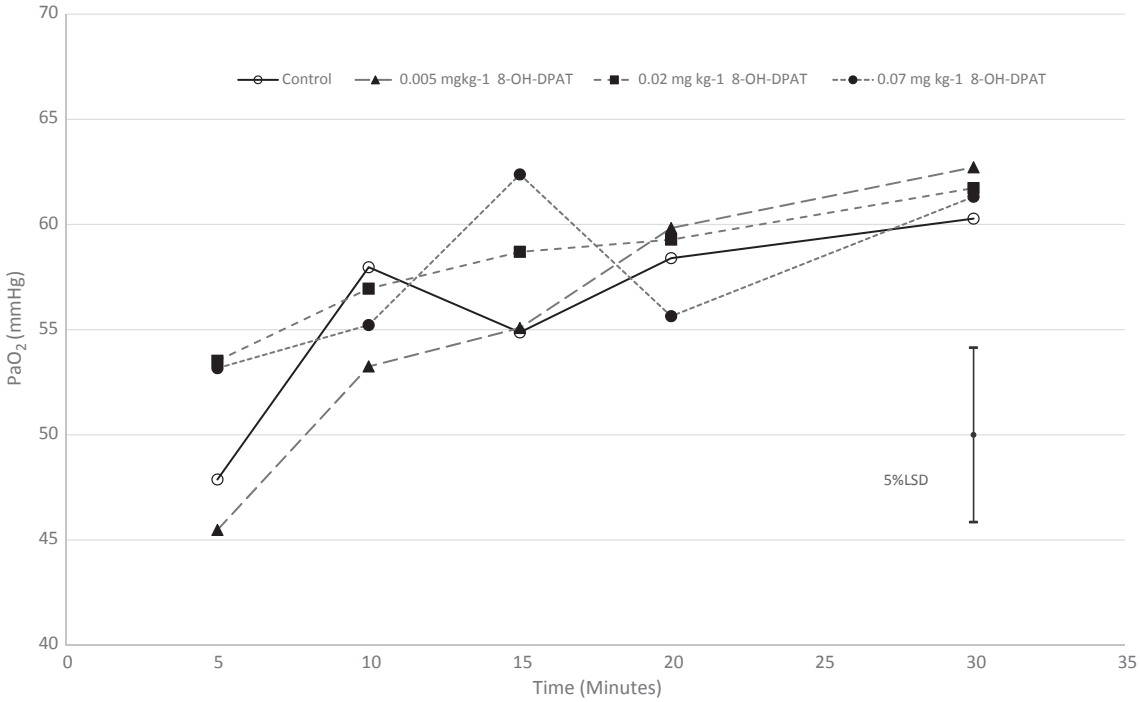

Figure 2 Effect of R-8-OH-DPAT treatment on PaO_2 (mmHg) of etorphine-immobilized impala ($n = 6$) over time. Control, low dose (0.005 mg kg^{-1}), medium dose (0.05 mg kg^{-1}), high dose (0.07 mg kg^{-1}). Error bars presented as least significant differences (5% LSD). A difference between the means larger than the LSD is considered significant.

In addition, the high-dose treatment group developed the most severe hypercapnia and hypoxia out of all the treatment groups. It is, therefore, concluded that although the f_R of blesbok increased when R-8-OH-DPAT was added, this did not translate into better ventilation. Equally, the addition of R-8-OH-DPAT did not appear to improve oxygen diffusion in the lungs as there were no clinical differences of PaO_2 values between the treatment groups. The significantly increased f_R of the medium-dosage treatment can possibly be explained as an artefact (type II error) associated with the small sample size. f_R is highly influenced by not only environmental factors such as stress and induction time but also by the person counting the respiration. The model did not allow for the identification of panting animals. This would have manifested as a higher f_R but a lower tidal volume.

It was previously speculated that the racemic 8-OH-DPAT and other serotonergic ligands improved pulmonary perfusion in goats and impala and thus facilitated better gas exchange (Meyer et al. 2006, 2010). This improved gas exchange was illustrated by a smaller A-a gradient (Herman et al. 2001; Meyer et al. 2010). R-8-OH DPAT had no effect on f_R or the A-a gradient of impala in this experiment, but it did attenuate the initial hypoxemia. Impala with the medium- and high-dose treatments at 5 minutes showed higher PaO_2 values than impala with low-dose and control treatments (Table 3 & Fig. 2).

The $PaCO_2$ values did not improve with any of the treatments. An explanation for a higher PaO_2 without improved ventilation or A-a gradient might be the reduction of the oxygen consumption through attenuation of the stress response due to the anxiolytic effects of R-8-OH-DPAT (Carli & Samanin, 1988; Picazo et al. 2000; Li et al. 2012; Żmudzka et al. 2018). Several studies have illustrated the beneficial effects of 8-OH-DPAT on HR of laboratory animals (Ngampramuan et al. 2008; Vianna & Carrive 2009; Horiuchi et al. 2011). In rats 0.05—0.1 mg kg^{-1} 8-OH-DPAT reduced the sympathetic response and mitigated the tachycardia induced by restraint stress (Ngampramuan et al. 2008; Vianna & Carrive 2009).

Evidence that the anxiolytic effect of R-8-OH-DPAT might have played a role in the cardiorespiratory events of impala at the start of the experiment would be the alleviation of the initial etorphine-induced tachycardia. Tachycardia was most severe after 5 minutes with the control and low-dose treatments (157 and 137 beats $minute^{-1}$, respectively) and less in the medium- and high-dose treatments (124 and 103 beats $minute^{-1}$, respectively; SED = 10.1; Table 3). Similar results were observed in a study on goats conducted by Meyer et al. (2006). When 8-OH-DPAT was injected with etorphine in goats, the HR (45 beats $minute^{-1}$) was lower than when etorphine was injected on its own (55 beats $minute^{-1}$) (Meyer et al. 2006).

Goats treated with 8-OH-DPAT developed a higher MAP (140 mmHg) than goats treated with etorphine only (120 mmHg) within the first 4—8 minutes after treatment. The MAP declined thereafter (Meyer et al. 2006). Similar results were observed in impala in the high-dose treatment with regards to mean values for DAP, which differed significantly from the control group at the 5 minutes time point, but DAP of the control group declined thereafter. Despite the statistically significant blood pressure changes, it is probable that the range in which the changes occurred were not of clinical significance.

It is apparent that the effect of R-8-OH-DPAT on physiological variables during etorphine immobilization differs between species. While R-8-OH-DPAT did not lead to any significant improvement of cardiovascular or blood-gas variables in blesbok, the positive effects of this serotonin agonist on etorphine-induced hypoxemia and tachycardia of impala from a dosage of 0.02 mg kg^{-1} and higher were apparent during the beginning of the immobilization.

Conclusion

The addition of R-8-OH-DPAT did not influence induction, immobilization or recovery of etorphine-immobilized impala or blesbok. In this experiment, it became apparent that the beneficial respiratory effects of R-8-OH-DPAT during immobilization with etorphine seem to be species-specific and cannot be generalized. This result, together with the possibility of serotonin toxicity at higher dosages, might not allow for the routine use of R-8-OH-DPAT during opioid-based wildlife immobilizations.

Species-specific effects of serotonergic ligands and the differences between the racemic 8-OH-DPAT and its R-enantiomer on respiratory and cardiovascular physiology of wildlife became apparent during the current experiment and warrant further investigation.

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Authors' contributions

SP: study design, manuscript, trial execution, data interpretation. LL and LM: study design, data interpretation and trial execution. KW: study design, manuscript and funding. RV-H: study design, manuscript, data interpretation, data analysis. JPR: study design, funding, data interpretation. ML: study design, data interpretation, funding, manuscript, trial execution, funding and main supervisor of SP.

Conflicts of interest

The research was funded by Wildlife Pharmaceuticals PTY LTD and Murdoch University. Other than that the authors declare no conflicts of interest. All authors have read the submitted version of the manuscript.

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