Research paper
Short title: Butorphanol effects in white rhinoceros

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

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Abstract

Objective

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

Study design

Randomized crossover study.

Animals

A group of six sub-adult male white rhinoceros.

Methods

Etorphine, or etorphine followed by butorphanol 12 minutes after recumbency, was administered intramuscularly [2.5 mg etorphine, 25 mg butorphanol (1000–1250 kg), or 3.0 mg etorphine, 30 mg butorphanol (1250–1500 kg)]. Sampling started at 10 minutes after initial recumbency, and was repeated at 5 minute intervals for 25 minutes. Arterial blood gases, limb muscle tremors, expired minute ventilation and respiratory frequency were measured at each sampling point. Calculated values included alveolar–arterial oxygen gradient, expected respiratory minute volume, tidal volume, physiological deadspace, oxygen consumption and carbon dioxide production.

Results

Etorphine administration resulted in an initial median (range) hypoxaemia [arterial partial pressure of oxygen 25.0 (23.0–28.0) mmHg], hypercapnia [arterial partial pressure of carbon dioxide 76.2 (67.2–81.2) mmHg], increased alveolar–arterial oxygen gradient [41.7 (36.6–45.1) mmHg, oxygen consumption [11.1 (10.0–12.0) L minute^{-1}] and muscle tremors. Butorphanol administration was followed by rapid, although moderate, improvements in arterial partial pressure of oxygen [48.5 (42.0–51.0) mmHg] and arterial partial pressure of carbon dioxide [62.8 (57.9 –75.2) mmHg]. In rhinoceros administered butorphanol, oxygen consumption $\dot{V}O_2$ [4.4 (3.6–5.1) L minute^{-1}] and carbon dioxide production $\dot{V}O_2$ [4.2 (3.8–4.4) L minute^{-1}] were lower than in those not administered butorphanol. Increased arterial oxygen tension was associated with lower oxygen consumption ($p = 0.002$) which was positively associated with lower muscle tremor scores ($p < 0.0001$).

Conclusions and clinical relevance

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

Keywords: butorphanol; etorphine; oxygen consumption; white rhinoceros

Introduction

Chemical capture is an essential tool in the management of free-ranging white rhinoceros (*Ceratotherium simum*) (Wenger et al. 2007). Etorphine, the opioid preferentially used in immobilization of rhinoceros, results in areflexia without total loss of consciousness, resulting from central nervous system depression within a few minutes following intramuscular (IM) administration (Portas 2004; Swan 1993). Etorphine is combined commonly with azaperone, a butyrophenone tranquillizer, which reduces induction times and opioid-associated hypertension (Portas 2004). Unfortunately, hypoxaemia, hypercapnia and acidaemia are well documented adverse effects associated with the use of etorphine and azaperone, and mortalities have been associated with immobilization in rhinoceros (Kock et al. 1995; Haw et al. 2015). Butorphanol, a mixed opioid agonist–antagonist, is administered intravenously (IV) in immobilized rhinoceros to mitigate these adverse

cardiorespiratory effects. However, inconsistent changes in blood gases following butorphanol administration have been reported. Different explanations for the effect of butorphanol in white rhinoceros, including improved ventilation or possible alterations in metabolic activity, have been proposed (Miller et al. 2013; Boardman et al. 2014; Haw et al. 2014; Buss et al. 2015). The objectives of this study were to determine the effects of etorphine on respiration in immobilized rhinoceros and changes that occur following IV administration of butorphanol. Cardiovascular effects of etorphine and IV butorphanol in these study rhinoceros have been previously reported (Buss et al. 2016).

Materials and methods

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

A group of six white rhinoceros, subadult (age, 5–6 years) males, were captured in Kruger National Park (23° 49′ 60 S, 31° 30′ 0 E; alt. 317m), South Africa, and habituated to captivity in holding pens over a period of 4 months. The study was a crossover design with two interventions allocated using computer generated random numbers with a 2 week washout period between treatments: 1) etorphine hydrochloride (9.8 mg mL^{-1}, M99, Elanco, Gauteng, South Africa) plus hyaluronidase (5000 i.u., Kyron Laboratories, Gauteng, South Africa), administered IM; and 2) etorphine hydrochloride plus hyaluronidase administered IM followed by butorphanol (50 mg mL^{-1}, Kyron Laboratories) administered IV. Doses were 2.5 mg etorphine, 5000 i.u. hyaluronidase, 25 mg butorphanol (1000–1250 kg), and 3.0 mg etorphine, 5000 i.u. hyaluronidase, 30 mg butorphanol (1250–1500 kg) (Haw et al. 2014; Buss et al. 2015). Azaperone was not included in the immobilizing drug combination because of potential confounding respiratory and cardiovascular effects (Portas 2004).

Etorphine plus hyaluronidase were administered using a 3.0 mL plastic dart with a 60 mm uncollared needle propelled from a compressed air rifle (DAN-INJECT, International S.A., South Africa). Once an animal could be safely handled, it was blindfolded and placed in sternal recumbency for 1 minute to facilitate initial instrumentation and subsequently rolled into lateral recumbency. The influence of variable induction times on physiological measurements was reduced by conducting a trial only if the rhinoceros became recumbent and could be safely handled within 15 minutes after darting (Haw et al. 2014). Recumbency was used as an indicator of immobilization level equivalency between trials. Data collection started 10 minutes after initial recumbency (t = 0), and was repeated at 5 minute intervals over a 25 minute study period. In those rhinoceros receiving treatment 2, butorphanol was administered IV at 2 minutes (t = 2) as this allowed for instrumentation of study animals and most closely approximated the time at which butorphanol is administered in field-immobilized rhinoceros. In treatment 1, rhinoceros were administered sterile saline at t = 2. At the end of each trial, all rhinoceros were weighed and administered naltrexone (40 mg mL^{-1}, Kyron laboratories) IV at 20 times the etorphine dose.

Expired minute ventilation (corrected for body temperature and saturated pressure; $\dot{V}E_{BTPS}$; L $minute^{-1}$) was measured via shortened equine endotracheal (ET) tubes (V KRUUSE I.D. 28, CAT. No. 282270, Jørgen Kruuse A/S, Denmark) inserted into each nostril with the cuffs inflated to create an airtight seal. A two-way Y-shape nonrebreathing valve (2730 Series; Hans Rudolph, Inc., OK, USA) was connected to each ET tube at the nares external margin which allowed inspired air to enter the tube and directed expired air to a PowerLab Exercise Physiology System (ML870B80; ADInstruments, NSW, Australia). The design and function of equipment collecting the expired air was tested in a pilot study to ensure that it did not impede respiratory function. Expired minute ventilation $(\dot{V}E_{BTPS})$ was determined via a respiratory flow head (MLT1000L) linked to a spirometer (ML140) and a gas mixing chamber (MLA245). The respiratory flow head had a 1000 L $minute^{-1}$ capacity and was calibrated daily according to manufacturer's instructions. Expired air temperature was recorded by a thermistor pod (ML309) in the mixing chamber.

Expired air was collected into a Douglas bag for 1 minute at the end of each sampling interval and analysed using a Cardiocap/5 (Datex-Ohmeda/GE Healthcare, Finland) for mixed-expired carbon dioxide pressure ($P\bar{E}CO_2$; mmHg) and expired oxygen fraction (FEO_2; %). The same monitor was used to analyse expired air from one of the ET tubes to determine end-tidal carbon dioxide pressure ($PE'CO_2$; mmHg) and oxygen fraction ($FÉO_2$; %), and measure respiratory frequency (*f*R). Body temperature (TB; °C) was measured using a rectal thermometer (BAT-12, Physitemp Instruments, NJ, USA).

A 22 gauge intravenous catheter was inserted into an auricular artery and blood samples collected into heparinized 1 mL syringes and immediately analysed using a portable blood gas analyser (iSTAT 1 Handheld Clinical Analyzer, Heska Corporation, CO, USA) and CG4+ cartridge (iSTAT CG4+ cartridges, Heska Corporation). The alveolar–arterial oxygen gradient [$P(A\text{-}a)O_2$] (mmHg) was calculated using the formula $FIO_2(PB - PH_2O) - PaCO_2 - PaO_2$, with inspired oxygen fraction (FIO_2; %) standardized to 20.9% and barometric pressure (PB; mmHg) measured by the portable blood gas analyser prior to each immobilization (PaO_2 = arterial partial pressure of oxygen; $PaCO_2$ = arterial partial pressure of carbon dioxide). Alveolar vapour pressure of saturated air (PH_2O; mmHg), at a specific TB, was determined using the formula 4.58 exp [(17.27 TB) / (237.3 + TB)] (Meyer et al. 2010). The expected respiratory minute ventilation ($\dot{V}EXP$; L $minute^{-1}$) in the rhinoceros prior to immobilization was estimated from body mass using the formula $0.518\ BM^{0.802}$ (Bide et al. 1997). Actual respiratory minute ventilation was considered to be equivalent to $\dot{V}E_{BTPS}$, which was divided by *f*R to calculate tidal volume (VT; L $breath^{-1}$).

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

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

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

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

Data analyses

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

Results

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

Treatment 1: etorphine

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

Table 1 Distribution of blood gases and respiratory parameters, median and interquartile range (25–75th percentile), at sampling periods 0, 5, 10, and 25 minutes in six captive male white rhinoceros (age 5–6 years) for two treatments: 1 [(etorphine intramuscularly (IM)]; and 2 [etorphine IM and butorphanol intravenously (IV)]

	Treatment[a]	0 minutes		5 minutes		10 minutes		25 minutes	
PaO_2 (mmHg) (kPa)	I	25.0 3.3	(23.0–28.0) (3.1–3.7)	25.0 3.3	(23.0–28.0) (3.1–3.7)	27.5 3.7	(23.0–29.0) (3.1–3.9)	26.0 3.5	(25.0–29.0) (3.3–3.9)
	II	25.5 3.4	(22.0–26.0) (2.9–3.5)	48.0 6.4	(42.0–50.0) (5.6–6.7)	48.5 6.5	(42.0–51.0) (5.6–6.8)	43.5 5.8	(38.0–46.0) (5.1–6.1)
$PaCO_2$ (mmHg) (kPa)	I	76.2 10.2	(67.2–81.2) (9.0–10.8)	71.1 9.5	(60.9–79.6) (8.1–10.6)	72.3 9.6	(66.9–81.8) (8.9–10.9)	78.5 10.5	(70.8–82.4) (9.4–11.0)
	II	81.7 10.9	(76.1–89.3) (10.1–11.9)	58.2 7.8	(54.7–68.4) (7.8–9.1)	62.8 8.4	(57.9–75.2) (7.7–10.0)	63.7 8.5	(62.8–65) (8.4–8.7)
$\dot{V}E_{BTPS}$ (L minute^{-1})	I	164.0	(126.6–182.2)	137.6	(102.5–153.7)	118.7	(88.5–130.6)	96.1	(66.8–101.4)
	II	151.4	(139.0–172.2)	153.0	(125.7–160.5)	89.5	(85.0–98.5)	83.0	(76.6–86.5)
*f*R (breaths minute^{-1})	I	10	(9–10)	9	(8–9)	7	(6–7)	6	(5–8)
	II	10	(8–10)	11	(10–13)	8	(6–8)	6	(6–7)
VT (L breath^{-1})	I	18.0	(14.1–21.5)	16.1	(14.0–16.7)	18.3	(17.7–18.7)	14.2	(12.7–15.5)
	II	16.8	(15.2–20.4)	12.2	(11.7–16.1)	11.9	(11.0–16.9)	13.4	(11.7–14.4)
$P(A–a)O_2$ (mmHg) (kPa)	I	41.7 5.6	(36.6–45.1) (4.9–6.1)	48.9 6.5	(39.0–53.3) (5.2–7.1)	44.3 5.9	(33.8–46.9) (4.5–6.3)	39.0 5.2	(33.6–44.1) (4.5–5.9)
	II	37.0 4.9	(33.3–41.5) (4.4–5.5)	38.9 5.2	(32.9–43.1) (4.4–5.7)	37.0 4.9	(30.7–39.3) (4.1–5.2)	36.7 4.9	(35.0–39.1) (4.7–5.2)
$\dot{V}D_{PHYS}$ (L minute^{-1})	I	59.5	(35.8–69.8)	42.1	(19.3–60.5)	39.5	(23.5–43.7)	35.6	(26.4–48.2)
	II	47.9	(46.9–50.9)	35.9	(17.5–52.2)	31.4	(21.9–41.2)	29.0	(26.1–34.5)
$\dot{V}O_2$ (L minute^{-1})	I	11.1	(10.0–12.0)	8.7	(7.7–9.8)	7.8	(5.8–8.6)	6.4	(3.7–7)
	II	10.9	(9.1–12.0)	6.8	(5.5–8.0)	4.4	(3.6–5.1)	4.2	(4.0–4.6)
$\dot{V}CO_2$ (L minute^{-1})	I	8.3	(7.8–11.4)	6.8	(5.9–7.8)	6.0	(4.9–7.1)	4.3	(3.5–5.3)
	II	9.3	(7.2–10.4)	6.9	(6.2–7.7)	4.2	(3.8–4.4)	3.7	(2.7–3.8)

PaO_2, arterial partial pressure oxygen; $PaCO_2$, carbon dioxide; $\dot{V}E_{BTPS}$, expired minute ventilation; f_R, respiratory rate; V_T, tidal volume; $P(A\text{-}a)O_2$, alveolar –arterial oxygen gradient; $\dot{V}D_{PHYS}$, physiological dead space; $\dot{V}O_2$, oxygen consumption; $\dot{V}CO_2$, carbon dioxide production.

[a] Treatment 1, etorphine IM (2.5 mg etorphine, 1000–1250 kg; 3.0 mg etorphine, 1250–1500 kg); Treatment 2, etorphine IM (2.5 mg etorphine 1000–1250 kg; 3.0 mg etorphine 1250–1500 kg) and butorphanol IV (25 mg butorphanol, 1000–1250 kg; 30 mg butorphanol, 1250–1500 kg).

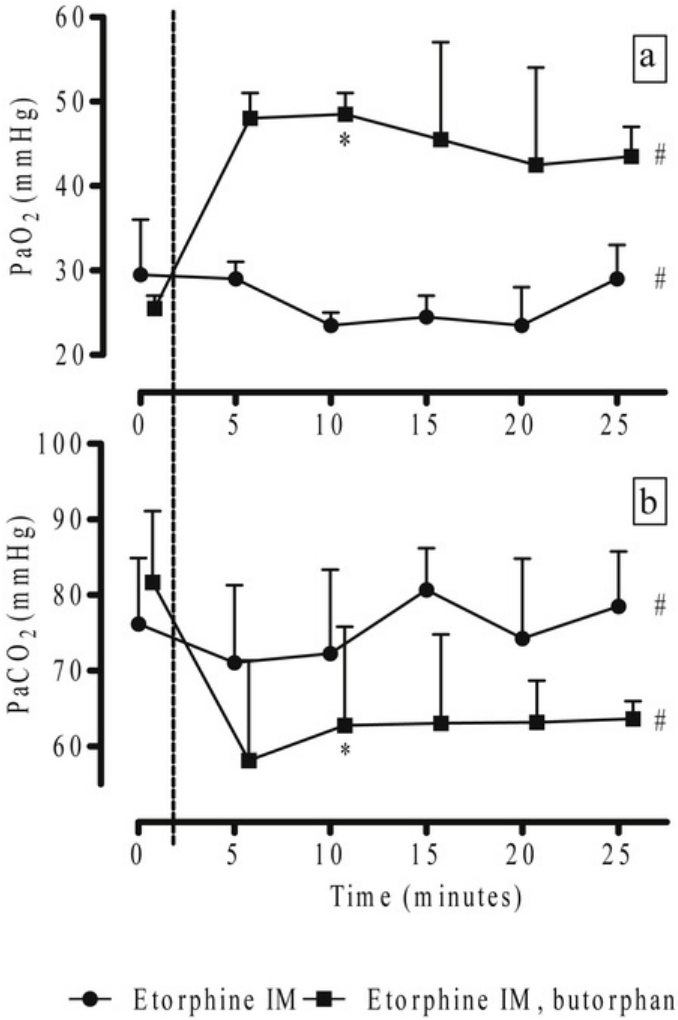

Figure 1 Median and interquartile range of arterial partial pressures of (a) oxygen (PaO_2) and (b) carbon dioxide ($PaCO_2$) at sampling periods 0, 5, 10, 15, 20 and 25 minutes in six captive male white rhinoceros (age, 5–6 years) for treatment 1 [(etorphine intramuscularly (IM)] or treatment 2 [etorphine IM and butorphanol intravenously (IV)]. The dashed line indicates the time at which butorphanol was administered. *, indicates a significant ($p < 0.05$) difference within treatment between t = 0 and t = 10, $^{+}$, indicates a significant ($p < 0.05$) difference within treatment between t = 10 and t = 25, $^{\#}$, indicates a significant ($p < 0.05$) difference in overall median values between treatments 1 and 2 from 5 to 25 minutes.

The median $\dot{V}EXP(t = 0)$ was 163 L minute^{-1}. $\dot{V}E_{BTPS}$ declined between t = 0–10 and t = 10–25; however, these changes was not statistically significant (Fig. 2). The *f*R decreased significantly ($p = 0.034$) over the first 10 minutes, but did not change between t = 10 and t = 25. No changes over time (t = 0–10, t = 10–25) were observed in VT, $P(A\text{-}a)O_2$ or $\dot{V}D_{PHYS}$.

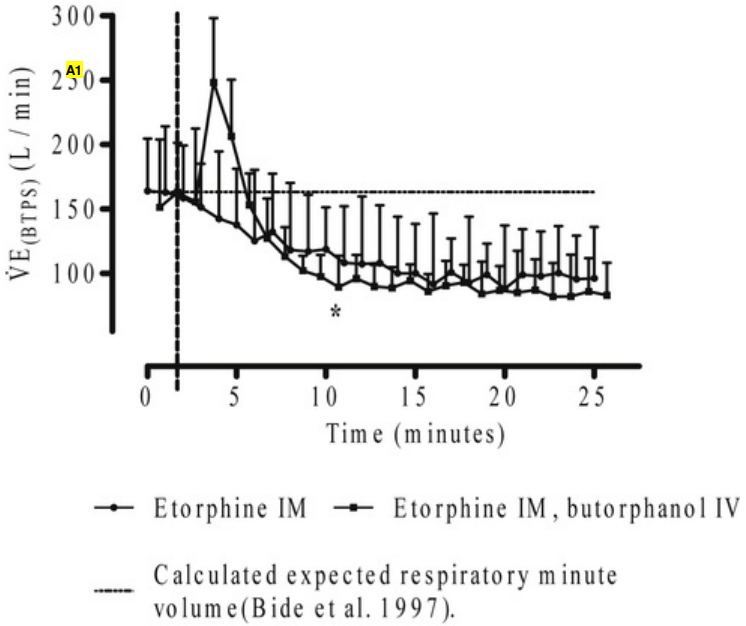

Figure 2 Median and interquartile range of expired minute ventilation ($\dot{V}E_{BTPS}$) measured at 1 minute intervals between 0 and 25 minutes in six captive male white rhinoceros (age, 5–6 years) for treatment 1 [(etorphine intramuscularly (IM)] or treatment 2 [etorphine IM and butorphanol intravenously (IV)]. The dashed line indicates the time at which butorphanol was administered. *, indicates a significant ($p < 0.05$) difference within treatment between t = 0 and t = 10; $^{+}$, indicates a significant ($p < 0.05$) difference within treatment between t = 10 and t = 25; $^{\#}$, indicates a significant ($p < 0.05$) difference in overall median values between treatments 1 and 2 from 5 to 25 minutes.

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

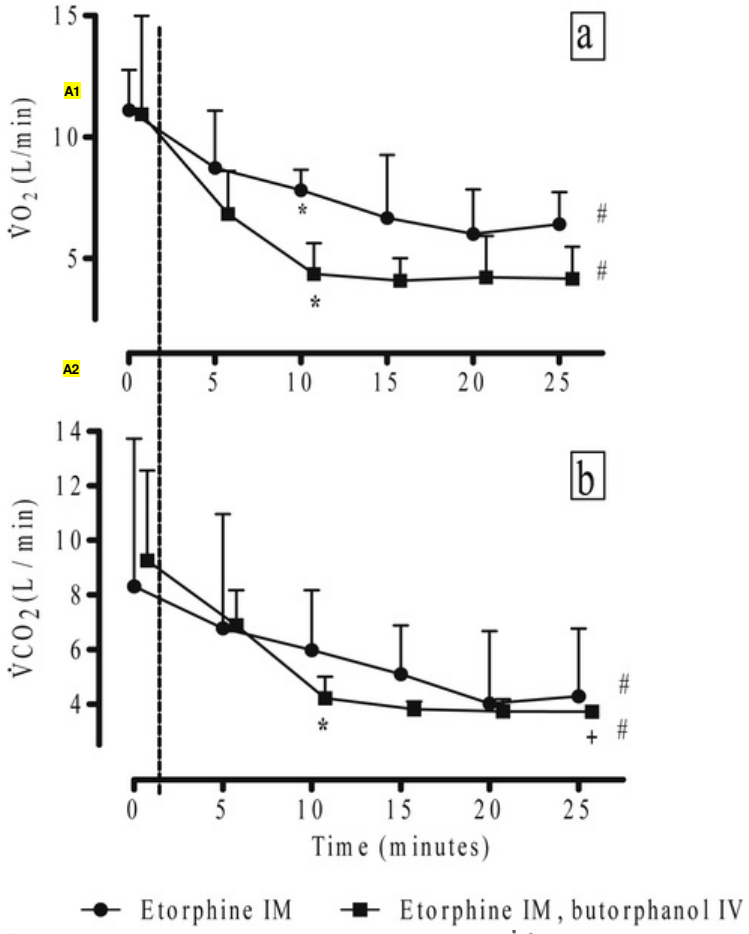

Figure 3 Median and interquartile range of (a) oxygen consumption ($\dot{V}O_2$) and (b) carbon dioxide production ($\dot{V}CO_2$) calculated for sampling periods 0, 5, 10, 15, 20 and 25 minutes in six captive male white rhinoceros (age, 5–6 years) for treatment 1 [(etorphine intramuscularly (IM)] or treatment 2 [etorphine IM and butorphanol intravenously (IV)]. The dashed line indicates the time at which butorphanol was administered. *, indicates a significant ($p < 0.05$) difference within treatment between t = 0 and t = 10; $^{+}$, indicates a significant ($p < 0.05$) difference within treatment between t = 10 and t = 25; $^{\#}$, indicates a significant ($p < 0.05$) difference in overall median values between treatments 1 and 2 from 5 to 25 minutes.

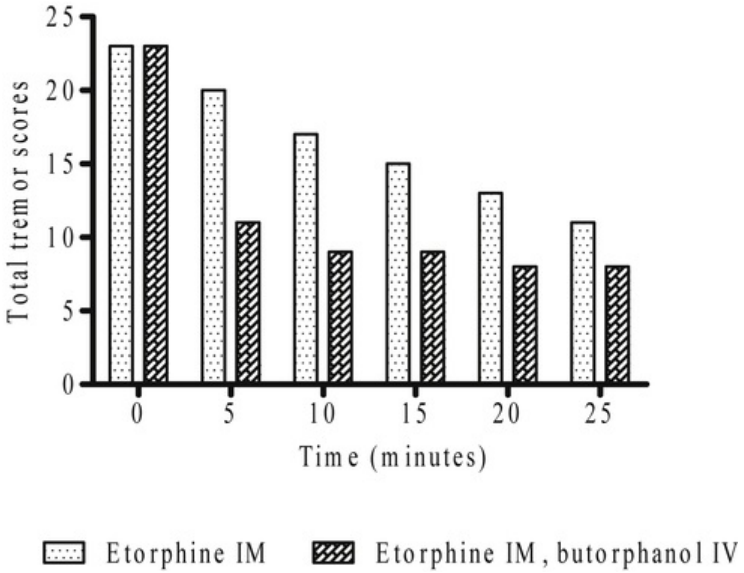

Figure 4 Muscle tremor scores at sampling periods 0, 5, 10, 15, 20 and 25 minutes were the sum of all the scores (1 to 5) at each time point in six captive male white rhinoceros (age, 5–6 years) for treatment 1 [(etorphine intramuscularly (IM)] or treatment 2 [etorphine IM and butorphanol intravenously (IV)].

Treatment 2: etorphine and butorphanol

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

Comparison of treatments 1 and 2

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

Table 2 Overall distribution of blood gases [arterial partial pressure oxygen (PaO_2) and carbon dioxide ($PaCO_2$), expired minute ventilation ($\dot{V}E_{BTPS}$), respiratory rate (fR), tidal volume (VT), alveolar –arterial oxygen gradient (P(A-a)O_2, physiological dead space ($\dot{V}D_{PHYS}$), oxygen consumption ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$)] and respiratory parameters, median and interquartile range (25–75th percentile), over the sampling period 5–25 minutes in six captive male white rhinoceros (age, 5–6 year) for two treatments: 1 [(etorphine intramuscularly (IM)]; and 2 [etorphine IM and butorphanol intravenously (IV)]

[a]Treatment	1		2	
PaO_2 (mmHg) (kPa)	26 3.5	(23–29) (3.1–3.9)	42.5 5.7	(30.5–48.5) (4.1–6.5)
$PaCO_2$ (mmHg) (kPa)	75.5 10.1	(67.4–82.3) (9.0–11.0)	64.3 8.6	(61.1–76.1) (8.1–10.1)
$VE_{(BTPS)}$ (L	105.9	(84.3–137.9)	90.0	(84.7–137.9)

minute^{-1})				
f_R (breaths minute^{-1})	7.0	(5–7)	7.0	(6–9)
V_T (L minute^{-1})	16.3	(14.0–18.3)	14.1	(11.8–16.2)
P(A–a)O_2 (mmHg) (kPa)	41.5 5.5	(34.1–45.8) (4.5–6.1)	36.8 4.9	(33.2–40.3) (4.4–5.4)
$\dot{V}O_2$ (L minute^{-1})	7.3	(5.6–8.7)	4.8	(4.1–6.8)
$\dot{V}$ CO_2 (L minute^{-1})	5.8	(4.2–7.2)	4.1	(3.8–6.3)
EDO				

[a] Treatment 1, etorphine IM (2.5 mg etorphine, 1000–1250 kg; 3.0 mg etorphine, 1250–1500 kg); Treatment 2, etorphine IM (2.5 mg etorphine 1000–1250 kg; 3.0 mg etorphine 1250–1500 kg) and butorphanol IV (25 mg butorphanol, 1000–1250 kg; 30 mg butorphanol, 1250–1500 kg).

Discussion

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

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

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

The initial median fR (10 breaths minute^{-1}) was substantially lower than the rate (16–23 breaths minute^{-1}) reported for standing unrestrained captive white rhinoceros (Citino and Bush 2007). These values suggest that $\dot{V}E_{BTPS}$ was maintained by an increase in VT in the immobilized rhinoceros. In the study rhinoceros, the increased VT may have been a compensatory response to hypoxaemia and/or, hypercapnia. It is also possible that initial sympathetic stimulation associated with darting influenced respiratory minute ventilation (Heistad et al. 1972).

In rhinoceros immobilized only with etorphine, hypoxaemia and hypercapnia did not change significantly over the 25 minute study interval. Although median $\dot{V}E_{BTPS}$ also did not change significantly, the initial and final values of 164 and 96 L minute^{-1}, respectively, may reflect a clinically relevant reduction in minute volume. A decrease in $\dot{V}E_{BTPS}$ is contradictory to the expected response to hypercapnia and hypoxia (Pattinson, 2008). Opioids suppress the ventilatory response to hypercapnia and hypoxia through reduced excitation of chemosensory neurons and chemoreceptor bodies (Yaksh & Wallace 2011). It is possible that etorphine receptor binding increased over time, causing further depression of respiratory rhythmogenesis resulting in hypopnoea; however, it is likely that peak respiratory perturbations had been reached at initial sample collection in the immobilized rhinoceros at approximately 20 minutes post-dart (Yaksh & Wallace 2011). The decrease in $\dot{V}E_{BTPS}$ may have been because of an opioid-induced diminished hypoxic drive associated with a lowered set-point. An arterial oxygen tension of 25 mmHg in immobilized rhinoceros should have stimulated an increase in ventilation, which was not observed. In opioid-immobilized rhinoceros, the hypothetically lowered hypoxic threshold would have resulted in a decreased respiratory stimulus at $PaO_2 > 25$ mmHg with a subsequent fall in ventilation and limited changes in PaO_2 (Pattinson 2008).

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

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

our findings of a strong correlation between $\dot{V}O_2$ and muscle tremor scores in immobilized rhinoceros.

The overall decrease in $\dot{V}E_{BTPS}$ over time in treatment 2 supports the hypothesis that IV administration of butorphanol in etorphine-immobilized rhinoceros does not improve respiratory minute ventilation. The $\dot{V}E_{BTPS}$ increased for a few minutes following the administration of butorphanol then subsequently decreased, similar to treatment 1, so overall there was no significant difference between the two treatments. The $\dot{V}D_{PHYS}$ was also similar between treatments, suggesting changes in deadspace ventilation did not influence blood gases following butorphanol administration. The P(A-a)O_2 was generally lower in treatment 2 compared to treatment 1, but an initial lower value and no significant change following butorphanol administration, suggests that this finding is of limited clinical importance. Further investigations are needed to confirm the consistency and significance of this difference between treatments as changes in alveolar ventilation: perfusion ratios, shunting, and gas diffusion rates across alveolar–capillary membranes could alter arterial blood gases (Haw et al. 2014; West 2008).

The results of this study suggest that the improvements in PaO_2 and $PaCO_2$, following butorphanol administration in etorphine-immobilized white rhinoceros, arose from changes in metabolic oxygen consumption associated with decreased muscle tremors. An inverse correlation between PaO_2 and $\dot{V}O_2$, and a positive correlation between $\dot{V}O_2$ and muscle tremor scores, suggest that these three variables are interrelated. We propose that an increase in PaO_2 following IV butorphanol administration is a result of decreased metabolism associated with reduced muscle tremors. Carbon dioxide production followed a similar trend to that of oxygen consumption which further supports the hypothesis that blood gas changes following butorphanol administration result from reduced metabolic activity. In shivering humans recovering from induced hypothermia, it has been shown that carbon dioxide production and oxygen consumption are positively associated with muscular activity and metabolic rate (Ralley et al. 1988). A reduction in muscle tremors associated with butorphanol administration may be mediated through antagonism of etorphine-induced sympathetic nervous system activity. Muscle tremors in immobilized rhinoceros are significantly associated with increased plasma catecholamine concentrations (De Lange 2015). The muscle tremors did not typify opioid-induced changes in locomotion often observed during induction in white rhinoceros. An alternative explanation for the improved arterial blood gases following butorphanol administration may be increased pulmonary perfusion and reduction in $\dot{V}/\dot{Q}$ inequality rather than changes in oxygen consumption (Wagner 2008). However, heart rate and systemic blood pressure decreased in etorphine-immobilized rhinoceros following butorphanol administration, suggesting a decrease in cardiac output (Buss et al. 2016).

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

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

Conclusion

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

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Authors contributions

PB: project designed and implementation, data collection, collation and analyses, and preparation of manuscript. MM: project implementation, including data collection, and preparation of manuscript. AF: project design and implementation, including data collection, and preparation of manuscript. AH: project implementation, including data collection, and preparation of manuscript. ES: statistical analysis of data and preparation of manuscript. FOP: project design, statistical analysis of data and preparation of manuscript. LM: project design, project implementation, including data collection, and preparation of manuscript.

Conflict of interest statement

There are no conflicts of interests by the authors.

Appendix A. Muscle tremor scores. Criteria for subjectively scoring muscle tremors

Degree of muscle tremor
Level 5 Severe tremors – resulting in whole body and head movement
Level 4 Moderate tremors – resulting in severe shoulder, chest, leg and foot movement
Level 3 Slight tremors – resulting in minor shoulder, chest and severe leg and foot movement
Level 2 Mild tremors – resulting in minor leg and foot movement
Level 1 No visible tremors

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