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Effects of Butorphanol on Respiration in White Rhinoceros (*Ceratotherium simum*) Immobilized with Etorphine-Azaperone

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ABSTRACT: This article reports on respiratory function in white rhinoceros (*Ceratotherium simum*) immobilized with etorphine-azaperone and the changes induced by butorphanol administration as part of a multifaceted crossover study that also investigated the effects of etorphine or etorphine-butorphanol treatments. Six male white rhinoceros underwent two immobilizations by using 1) etorphine-azaperone and 2) etorphine-azaperone-butorphanol. Starting 10 min after recumbency, arterial blood gases, limb muscle tremors, expired minute ventilation, and *f*R were evaluated at 5-min intervals for 25 min. Alveolar to arterial oxygen gradient, expected respiratory minute volume, oxygen consumption, and carbon dioxide production were calculated. Etorphine-azaperone administration resulted in hypoxemia and hypercapnia, with increases in alveolar to arterial oxygen gradient, oxygen consumption, and carbon dioxide production, and a decrease in expired minute ventilation. Muscle tremors were also observed. Intravenous butorphanol administration in etorphine-azaperone–immobilized white rhinoceros resulted in less hypoxemia and hypercapnia; a decrease in oxygen consumption, carbon dioxide production, and expired minute ventilation; and no change in the alveolar to arterial oxygen gradient and rate of breathing. We show that the immobilization of white rhinoceros with etorphine-azaperone results in hypoxemia and hypercapnia and that the subsequent intravenous administration of butorphanol improves both arterial blood oxygen and carbon dioxide partial pressures.

Key words: Azaperone, butorphanol, etorphine, metabolism, white rhinoceros.

INTRODUCTION

Etorphine-azaperone is preferentially used in the immobilization of free-ranging white rhinoceros (*Ceratotherium simum*) for management purposes (La Grange et al. 2016). Etorphine, a high-potency opioid, enables a sufficient dose for the immobilization of a rhinoceros to be delivered intramuscularly (IM) by dart; however, a major side effect is depression of ventilation, resulting in hypoxemia, hypercapnia, and acidemia (Buss et al. 2018). Alternative potent opioids including thiafentanil or carfentanil are not routinely used in the immobilization of free-ranging white rhinoceros. Etorphine is seldom administered on its own and is usually combined with azaperone, a butyrophenone tranquilizer, to shorten induction time from drug administration to the animal becoming immobilized (Buss et al. 2022). Azaperone modifies animal behavior, primarily by dopamine receptor blockade (Leysen and Gommeren 2008), and may influence breathing modulation through central and peripheral receptors (Hsiao et al. 1989). Although etorphine-azaperone is commonly used to immobilize rhinoceros, ventilatory perturbations pose significant mortality risks, especially in already compromised individuals. Therefore, it is essential to investigate the pathophysiology of these negative physiologic

consequences to facilitate the development of improved chemical capture procedures (Miller et al. 2013; Boardman et al. 2014; Haw et al. 2014).

In the past 10 yr, butorphanol has been commonly administered to immobilized rhinoceros in the belief that it antagonizes depression of ventilation caused by various anaesthetic agents (Haw et al. 2014; Wenger et al. 2007). As part of a larger study, Buss et al. (2018) showed that administration of butorphanol intravenously (IV) in etorphine-immobilized rhinoceros, at a ratio of 10 mg of butorphanol to 1 mg of etorphine, lessened the marked opioid-induced hypoxia and hypercapnia; this effect was mediated by decreased oxygen consumption and carbon dioxide production associated with reduced muscle tremoring.

The aim of this study was to investigate the effects of butorphanol in white rhinoceros immobilized with etorphine-azaperone, the effects of which had not been investigated previously. It was hypothesized that the administration of butorphanol to etorphine-azaperone–immobilized white rhinoceros would decrease metabolic rate, resulting in decreased hypoxemia and hypercapnia.

MATERIALS AND METHODS

Study design

The study had ethical approval from the South African National Parks (SANParks) Animal Use and Care Committee (reference 14-2) and the University of the Witwatersrand Animal Research Ethics Committee (reference 2014/15/C). Management of the white rhinoceros was conducted according to the SANParks standard operating procedures for the capture, transportation, and maintenance in holding facilities of wildlife.

Six subadult (5- to 6-yr-old) male white rhinoceros were captured in the Kruger National Park, South Africa, in June 2014 and habituated to captivity in holding pens over a period of 4 mo. The number of study animals was limited by welfare considerations and logistical challenges associated with adapting and managing wild-caught rhinoceros in captivity. White rhinoceros were administered the following two treatments from October 2014 to February 2015: 1) etorphine (9.8 mg/mL, M99, Elanco, Gauteng, South Africa)-azaperone (40 mg/mL, Janssen Pharmaceutical Ltd., Halfway House, South Africa) IM and 2) etorphine-azaperone IM followed by butorphanol (50 mg/mL, Kyron Laboratories, Gauteng, South Africa) IV. Both treatments included hyaluronidase (5,000 IU, Kyron Laboratories). Doses were 2.5 mg of etorphine, 37.5 mg of azaperone, and 25 mg of butorphanol for 1,000- to 1,250-kg white rhinoceros and 3.0 mg of etorphine, 45 mg of azaperone, and 30 mg of butorphanol for 1,250- to 1,500-kg white rhinoceros (Haw et al. 2014; Buss et al. 2016). An additional two treatments, etorphine alone and etorphine-butorphanol, were part of a larger study in the same white rhinoceros (Buss et al. 2018).

A multifaceted crossover study design was used in which a study white rhinoceros was randomly allocated one of the two treatments and 2 wk later it was administered the second treatment. Food and water were removed from the white rhinoceros in the late afternoon, and it was immobilized in an outdoor holding facility early the next morning when environmental temperatures were cool.

Drug administration and sample collection

Etorphine-azaperone was 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). Induction time was measured as the time from dart placement to the animal becoming recumbent with its body on the ground either in a sternal or lateral position. The immobilized rhinoceros was blindfolded and initially placed in sternal recumbency for 1 min and then subsequently rolled randomly into right or left lateral recumbency to facilitate instrumentation. The influence of variable induction times on physiologic measurements was reduced by conducting a trial only if the rhinoceros became recumbent and could be safely handled within 15 min after darting (Buss et al. 2018). As part of the larger study, each rhinoceros was immobilized four times; however, two rhinoceros did not become immobilized within this time limit and the interventions were repeated after a 2-wk washout period. These two animals were immobilized five times. Recumbency was used as an indicator of immobilization level equivalency between trials. Data collection started 10 min after initial recumbency ($t = 0$) and was repeated at 5-min intervals over a 25-min study

period. Butorphanol was administered IV into an auricular vein by using a 1-mL syringe and 20-gauge needle (Thermo Fisher Scientific, Randburg, South Africa) at 2 min (t = 2) because this allowed for initial data collection at t = 0 and most closely approximated the time at which butorphanol is administered in field-immobilized rhinoceros. In rhinoceros not receiving butorphanol, sterile saline was administered IV at t = 2. At the end of each trial, all rhinoceros were weighed, administered naltrexone (40 mg/mL, Kyron laboratories) IV at 20 times the etorphine dose (in milligrams), and observed until standing without any residual etorphine-induced clinical effects. The rhinoceros were monitored for a further 2 wk as described by Miller et al. (2016) to ensure sufficient food intake; appropriate fecal volume, color, and consistency; and normal demeanor.

Once a rhinoceros was instrumented, respiratory functions, oxygen consumption, arterial blood gases, and muscle tremor scores were evaluated. Respiratory physiologic values were calculated as described previously (Buss et al. 2018). Expired minute ventilation corrected for body temperature and saturated pressure ($\dot{V}E_{BTPS}$ in liters per minute) was measured by redirecting expired air through a PowerLab exercise physiology system (ADInstruments, Castle Hill, New South Wales, Australia) by using modified equine endotracheal (ET) tubes (Jørgen Kruuse A/S, Langeskov, Denmark) inserted into each nostril with the cuffs inflated to create an airtight seal; a 1,000-L/min capacity respiratory flow head linked to a spirometer (ML140); and a 4.7-L capacity gas mixing chamber (all from ADInstruments). A two-way Y-shape nonrebreathing valve (2730 Series; Hans Rudolph, Inc., Shawnee, Kansas, USA) was attached to the end of each ET tube to allow inspiration of fresh air and expiration through the PowerLab system. Expired air temperature was recorded by a thermistor pod (ADInstruments) in the mixing chamber. The respiratory flow head was calibrated daily according to manufacturer's instructions.

Expired air was collected in a Douglas bag (Harvard Apparatus, Holliston, UK) for 1 min during each sampling interval and analyzed using a multiparameter monitor (Cardiocap/5, Datex-Ohmeda, GE Healthcare, Helsinki, Finland) for mixed-expired carbon dioxide pressure ($P\overline{E}CO_2$, in millimeters of mercury) and mixed-expired oxygen percentage (FEO_2 in percent). Expired air from one of the ET tubes was analyzed using the same monitor to determine end-tidal carbon dioxide pressure ($PE'CO_2$ in millimeters of mercury), oxygen fraction ($FE'O_2$ in percent), and respiratory frequency (*f*R). Body temperature (T in Celsius) was measured using a rectal digital thermometer (BAT-12, Physitemp Instruments, Clifton, New Jersey, USA).

Arterial blood samples were collected into 1-mL heparinized syringes from a 22-gauge IV catheter placed into an auricular artery, and immediately analyzed using a portable blood gas analyser (i-STAT 1 handheld clinical analyzer, Heska Corporation, Loveland, Colorado, USA) and CG4+cartridge (i-STAT CG4+cartridges, Heska Corporation). Alveolar to arterial oxygen gradient [$P(A\text{-}a)O_2$ in millimeters of mercury] was calculated using the formula $F_IO_2(P_B-P_{H2O})-(PaCO_2/RQ)-PaO_2$, where PaO_2 is arterial partial pressure of oxygen, $PaCO_2$ is arterial partial pressure of carbon dioxide, and RQ is respiratory quotient. Inspired fraction for oxygen was assumed to be $F_IO_2 = 20.9\%$ and barometric pressure (P_B in millimeters of mercury) was measured by the portable blood gas analyzer before each immobilization. The RQ is unknown for white rhinoceros and was assumed to be 1. Alveolar vapor pressure of saturated air (P_{H2O} in millimeters of mercury), at a specific T, was determined using the formula 4.58 exp [(17.27 T)/(237.3+T) according to Meyer et al. (2010) and expected respiratory minute ventilation ($\dot{V}_{EXP}$ in liters per minute) before immobilization was estimated using the formula 0.518 (body mass$^{0.802}$; Bide et al. 1997). Actual respiratory minute ventilation was equivalent to $\dot{V}E_{BTPS}$, which was divided by fR to calculate tidal volume (VT in liters per breath).

The Enghoff modified Bohr's equation $((PaCO_2-P\overline{E}CO_2)/PaCO_2)\times VT$ was used to determine physiologic dead space ($\dot{V}D_{PHYS}$ in liters per breath; Tusman et al. 2012). The calculated $\dot{V}D_{PHYS}$ was corrected by 300 mL/breath for the volume of the two ET tubes extending beyond the rhinoceros' nostrils.

Oxygen consumption ($\dot{V}O_2$ in liters per minute) was calculated as describe previously (McArdle et al. 1986) as $\dot{V}O_2 = (FIO_2-FEO_2)/100\times(\dot{V}E_{STPD})$, where FEO_2 is expired oxygen fraction and $\dot{V}E_{STPD}$ is expired minute ventilation at standard temperature and dry pressure. The $\dot{V}E_{BTPS}$ was multiplied by (273/310)((PB−47)/760) to convert from BTPS to standard temperature and pressure and dry (STPD; West 2008). The Haldane transformation was used to correct the inspired oxygen volume, that is,

TABLE 1. Muscle tremor scores: criteria for subjectively scoring muscle tremors in chemically immobilized white rhinoceros (*Ceratotherium simum*).

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

$\dot{V}O_2 = \dot{V}E_{STPD}(FIO_2((1-(FEO_2+FECO_2)/1-(FIO_2+FICO_2))-FEO_2)$ according to McArdle et al. (1986), because inspired and expired minute ventilations were not equivalent (depending on the RQ), and $\dot{V}E_{STPD}$ was used to determine both FIO_2 and FEO_2. The inspired fraction for carbon dioxide (F_ICO_2) was assumed to be 0.03%. Carbon dioxide production ($\dot{V}CO_2$ in liters per minute) was calculated as the product of $\dot{V}E_{STPD}$ and the difference between expired and inspired carbon dioxide fractions $[\dot{V}E_{STPD}(FECO_2-F_ICO_2)]$ according to McArdle et al. (1986).

Muscle tremor scores, especially of the limbs, head, and shoulder, were subjectively evaluated by a single observer at each time point by using set criteria (Table 1). Scores ranged from 1 (no visible tremors) to 5 (severe tremors). Total muscle tremor scores were calculated as the sum of all scores per time point for that treatment.

Interpretation of the physiologic parameters resulting from etorphine-azaperone and etorphine-azaperone-butorphanol was facilitated by comparing results with those from the same rhinoceros administered etorphine alone or etorphine-butorphanol as part of the multifaceted crossover study (Buss et al. 2018). Equivalent drug doses were administered to all study animals under the same conditions, and the resulting physiologic effects were evaluated using identical methods (Buss et al. 2018).

Data analyses

We used STATA 14 (StatCorp, College Station, Texas, USA) for statistical analyses. Descriptive statistics were calculated to assess data distribution for each treatment at different sampling points. Because of the small sample size ($n = 6$), nonparametric statistical tests were used to compare median blood gas and respiratory values at specific sampling points within each treatment. For an initial and exploratory phase, the Kruskal-Wallis test was used to assess whether median values for blood gases and respiratory variables differed over sampling points. Median values were then compared between t = 0 and t = 10 as well as t = 0 and t = 25, and t = 10 and t = 25 by using the Wilcoxon signed rank test. To confirm that no further changes occurred after 10 min, linear regression (using ranks) was used to assess changes in blood gases and respiratory parameters after 10 min by using t = 10 as the reference value. Correlations between blood gases, respiratory parameters, and muscle tremor scores were evaluated using linear regression while controlling for time and repeated measures. Statistical significance was set at $P<0.05$ for all statistical tests.

RESULTS

All rhinoceros except two became recumbent within 15 min of etorphine-azaperone administration by dart. Prolonged inductions in the two animals were because of inaccurate dart placement resulting in slow drug absorption. These rhinoceros were reimmobilized without further complications after a 2-wk washout period. Immobilized rhinoceros were instrumented within 10 min of becoming recumbent and remained immobilized for the study period; no addition doses of drugs were necessary.

Treatment: etorphine-azaperone

Etorphine-azaperone administration resulted in an initial median $PaO_2 = 24.0$ mmHg, $PaCO_2 = 65$ mmHg, $P(A\text{-}a)O_2 = 53$ mmHg, $\dot{V}E_{BTPS} = 70$ L/min, $\dot{V}O_2 = 4.4$ L/min, and $\dot{V}CO_2 = 3.2$ L/min, along with muscle tremors. There were no significant changes in median PaO_2, $PaCO_2$, $P(A\text{-}a)O_2$, and $\dot{V}D_{PHYS}$ over time (t = 0 to t = 25; Fig. 1); however, declines in median $\dot{V}O_2$, $\dot{V}CO_2$, V_T, and $\dot{V}E_{BTPS}$ between t = 0 and t = 25 were significant ($P = 0.028$; Figs. 2 and 3). Median fR increased significantly ($P = 0.030$) from t = 10 to t = 25 (Table 2).

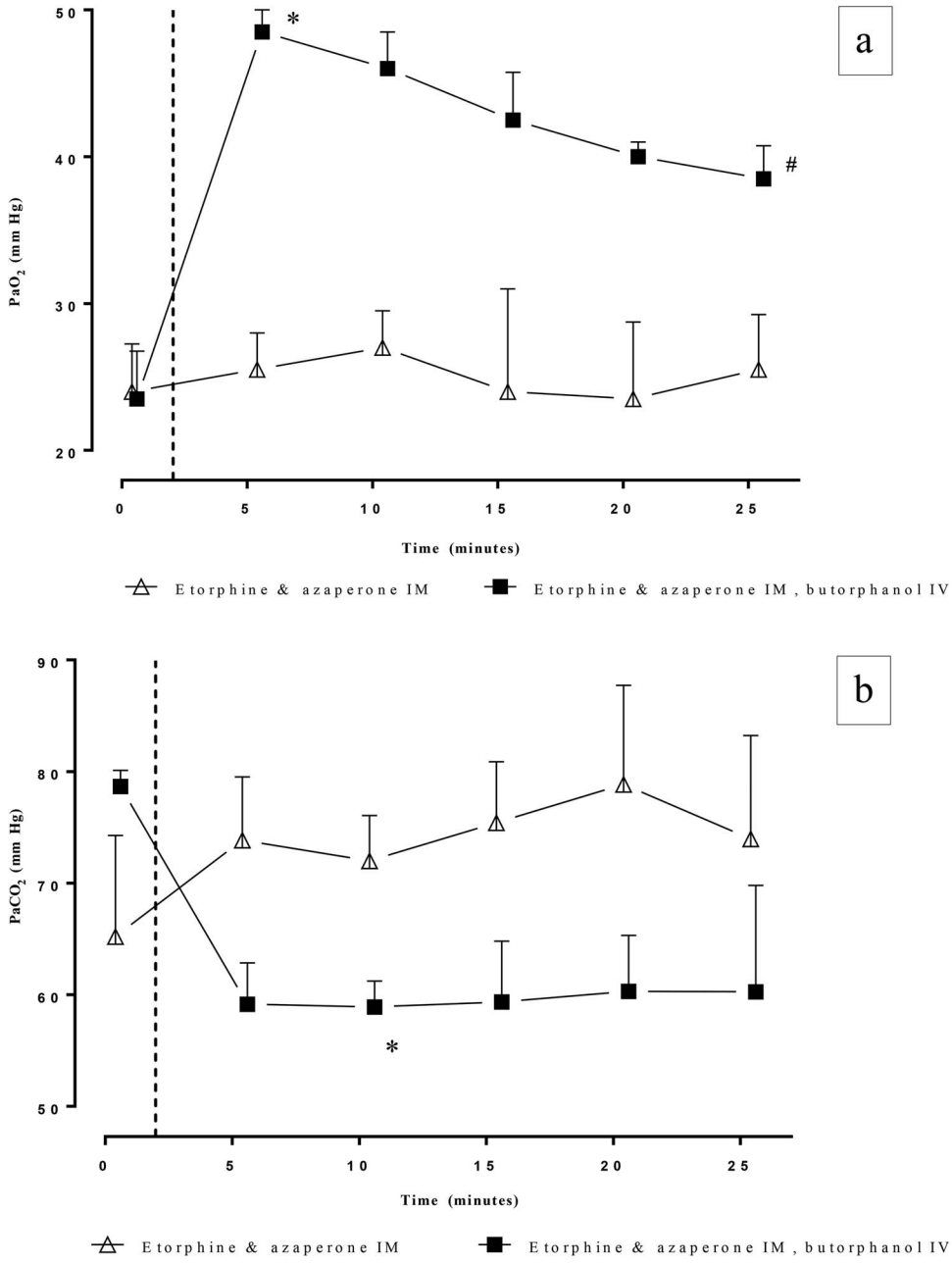

FIGURE 1. Median and interquartile range of arterial partial pressures of (a) oxygen (PaO_2) and (b) carbon dioxide ($PaCO_2$) at sampling periods 0, 5, 10, 15, 20, and 25 min in six 5- to 6-yr-old captive male white rhinoceros (*Ceratotherium simum*) for treatment etorphine-azaperone intramuscularly (IM) or treatment etorphine-azaperone IM plus butorphanol intravenously (IV). Arterial blood samples collected anaerobically from the auricular artery. The study was conducted at Skukuza, Kruger National Park, South Africa (elevation, 281 m; atmospheric pressure, 734–737 mmHg; environmental temperature, 23.4–29 C); rhinoceros rectal body temperature, 35.8–37.5 C. The dashed line indicates the time at which butorphanol was administered. The asterisk (*) indicates a significant ($P<0.05$) difference within treatment between t = 0 and t = 10. The pound symbol (#) indicates a significant ($P<0.05$) difference within treatment between t = 10 and t = 25. The infinity symbol (∞) indicates a significant ($P< 0.05$) difference with treatment between t = 0 and t = 25.

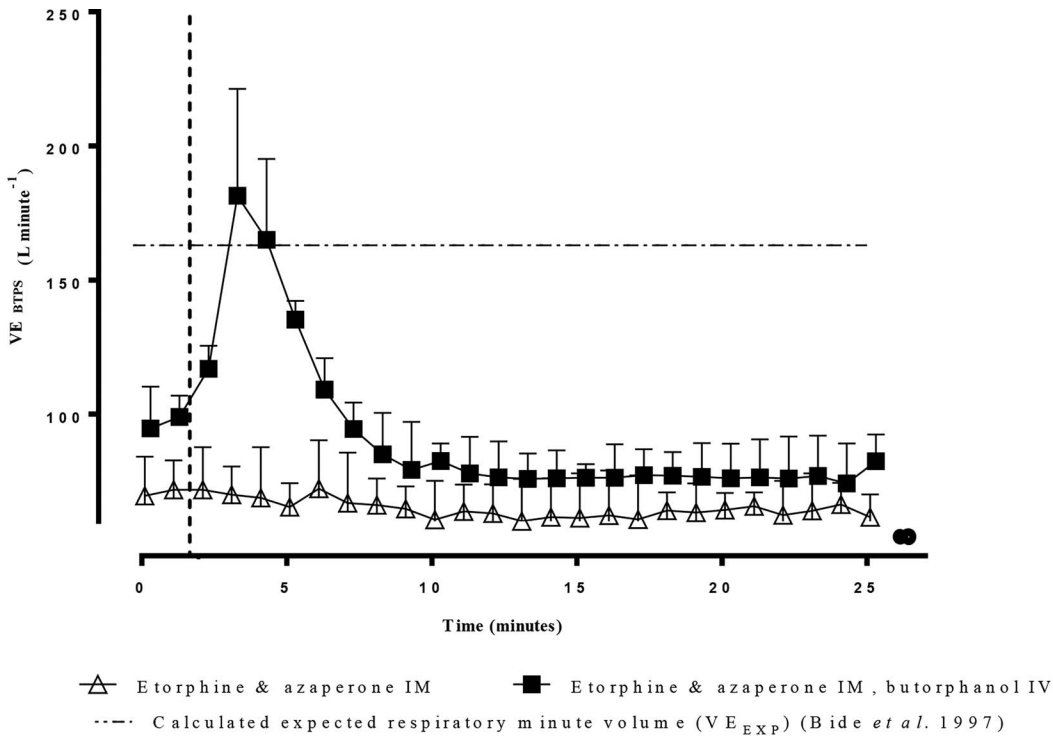

FIGURE 2. Median and interquartile range of expired minute ventilation ($\dot{V}E_{BTPS}$) measured at sampling periods 0, 5, 10, 15, 20, and 25 min in six 5- to 6-yr-old captive male white rhinoceros (*Ceratotherium simum*) for treatment etorphine plus azaperone intramuscularly (IM) or treatment etorphine plus azaperone IM and butorphanol intravenously (IV). Expired minute ventilation was measured by directing expired air through a PowerLab exercise physiology system by using modified equine endotracheal tubes place in each nostril. The study was conducted at Skukuza, Kruger National Park, South Africa (elevation, 281 m; atmospheric pressure, 734–737 mmHg; environmental temperature, 23.4–29 C); rhinoceros rectal body temperature, 35.8–37.5 C. The dashed line indicates the time at which butorphanol was administered. The asterisk (*) indicates a significant ($P<0.05$) difference within treatment between t = 0 and t = 10. The pound symbol (#) indicates a significant ($P<0.05$) difference within treatment between t = 10 and t = 25. The infinity symbol (∞) indicates a significant ($P<0.05$) difference with treatment between t = 0 and t = 25.

There was a positive association between $\dot{V}O_2$ and $\dot{V}CO_2$, with muscle tremor scores ($P<0.0001$, $r^2 = 0.56$ and $P = 0.0001$, $r^2 = 0.54$, respectively) that decreased over the trial period (Fig. 4). There was an inverse association between PaO_2 and $\dot{V}O_2$ ($P = 0.002$, $r^2 = 0.25$) and no significant association between $PaCO_2$ and $\dot{V}CO_2$ ($P = 0.370$, $r^2 = 0.02$).

Treatment: etorphine-azaperone-butorphanol

The administration of butorphanol IV at t = 2 in etorphine-azaperone–immobilized rhinoceros was associated with a significant increase in median PaO_2 ($P = 0.027$) and decrease in median $PaCO_2$ ($P = 0.028$) between t = 0 and t = 10. Between t = 10 and t = 25, PaO_2 decreased ($P = 0.027$) and there was no significant change in $PaCO_2$ (Fig. 1). The median $\dot{V}O_2$ and the $\dot{V}CO_2$ decreased between t = 0 and t = 10 ($P = 0.028$ and $P = 0.028$, respectively), with no further statistically significant changes from t = 10 to t = 25 (Fig. 3). There was a rapid but short-lived increase in median $\dot{V}E_{BTPS}$ from t = 2 to t = 5, but changes between t = 0 to t = 25 were not statistically different (Fig. 2). Median $P(A\text{-}a)O_2$, V_T, fR, and $\dot{V}D_{PHYS}$ did not change significantly over time (Table 2).

The $\dot{V}O_2$ and $\dot{V}CO_2$ were positively associated with muscle tremor scores ($P = 0.001$, $r^2 = 0.39$

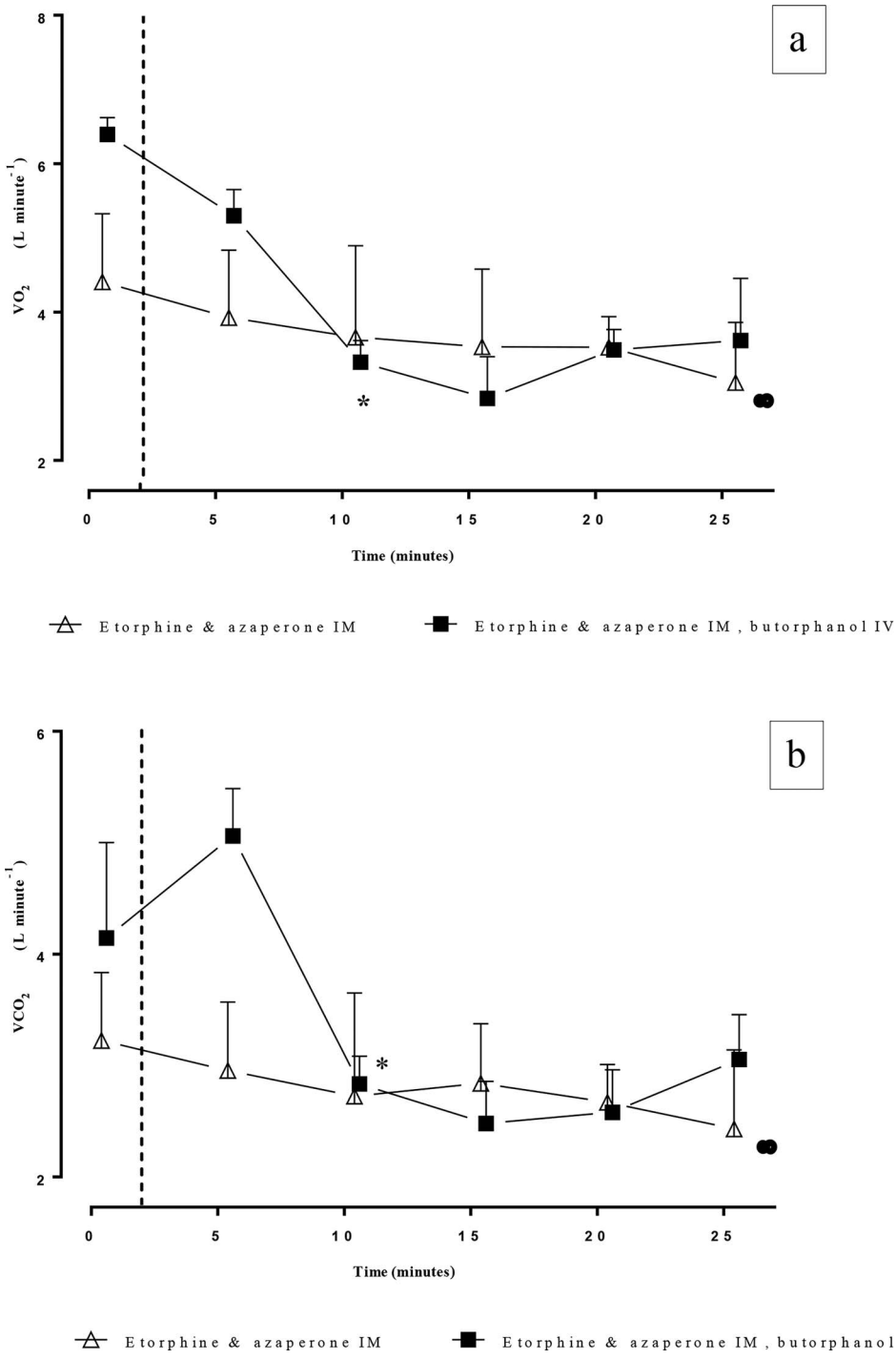

FIGURE 3. Median and interquartile range of (a) oxygen consumption ($\dot{V}O_2$) and (b) carbondioxide production ($\dot{V}CO_2$) calculated at sampling periods 0, 5, 10, 15, 20, and 25 min in six 5- to 6-yr-old captive male white rhinoceros (*Ceratotherium simum*) for treatment etorphine plus azaperone intramuscularly (IM) or treatment etorphine plus azaperone IM and butorphanol intravenously (IV). Oxygen consumption was calculated using the inspired oxygen fraction, mixed-expired oxygen percentage of expired air collected in a Douglas bag and analyzed using a Cardiocap/5 monitor, and expired minute ventilation was measured by directing expired air

and $P = 0.024$, $r^2 = 0.25$, respectively), which decreased rapidly between t = 0 and t = 5 and remained low for the rest of the immobilization period (Fig. 4). There was an inverse association between PaO_2 and $\dot{V}O_2$ ($P = 0.008$, $r^2 = 0.19$) and no significant association between $PaCO_2$ and $\dot{V}CO_2$ ($P = 0.928$, $r^2 = 0.0002$).

Table 3 shows the overall distribution of blood gas and respiratory values (from t = 5 to t = 25) for etorphine-azaperone and etorphine-azaperone-butorphanol treatments. The median PaO_2 was higher ($P<0.001$) and the median $PaCO_2$ was lower ($P<0.001$) in etorphine-azaperone-butorphanol–immobilized rhinoceros than in etorphine-azaperone–immobilized rhinoceros. Median f_R was significantly higher ($P<0.001$) and V_T was statistically lower ($P = 0.002$) in animals given butorphanol. There was no significant difference in overall median $P(A\text{-}a)O_2$, $\dot{V}O_2$, and $\dot{V}CO_2$ between treatments.

The rhinoceros in this study made uneventful recoveries and were standing within 2 min after naltrexone administration. Food consumption; quantity, consistency, and color of feces; and demeanor were not changed in any rhinoceros as the result of either of the treatments.

DISCUSSION

Hypoxemia and hypercapnia were of clinical concern in white rhinoceros immobilized with etorphine-azaperone, as reported previously in white rhinoceros immobilized with etorphine (Buss et al. 2018; Table 2); however, butorphanol administration provided clinically beneficial improvements in hypoxemia and hypercapnia in etorphine-azaperone–immobilized rhinoceros, similar to the effects of butorphanol in rhinoceros immobilized with etorphine alone (Buss et al. 2018). The improvements in PaO_2 and $PaCO_2$ after butorphanol administration did not appear to be a result of increased minute ventilation, because an initial increase was short lived. Positive associations between muscle tremor scores and $\dot{V}O_2$ and $\dot{V}CO_2$, which both decreased after butorphanol IV, indicate that these improvements in blood gases probably resulted from a decrease in metabolic activity.

An unexpected finding was that $\dot{V}E_{BTPS}$ did not change significantly after butorphanol administration in our etorphine-azaperone–immobilized rhinoceros compared with an increase in ventilation after butorphanol administration to the same rhinoceros immobilized with etorphine (Buss et al. 2018). The different outcome for the two interventions appeared to be associated with a lower $\dot{V}E_{BTPS}$ at t = 0 in etorphine-azaperone–immobilized rhinoceros. In the rhinoceros immobilized with etorphine-azaperone, there was an initial short-lived increase in $\dot{V}E_{BTPS}$ after butorphanol administration; however, the ventilation trends and values were similar to those in rhinoceros not receiving butorphanol for the remainder of the immobilization period.

Butorphanol administration in etorphine-azaperone–immobilized rhinoceros resulted in PaO_2 values that were inversely associated with $\dot{V}O_2$ and muscle tremor scores, supporting the theory that butorphanol-induced reduction in metabolic activity led to improvements in blood gas values (Buss et al. 2018). It has been previously shown that butorphanol administration decreased muscle tremor scores in etorphine-immobilized white rhinoceros, resulting in a rapid and significant decrease in both hypoxemia and hypercapnia (Buss et al. 2018); however, unlike etorphine-immobilized rhinoceros

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through a PowerLab exercise physiology system by using modified equine endotracheal tubes place in each nostril. The study conducted at Skukuza, Kruger National Park, South Africa (elevation, 281 m; atmospheric pressure, 734–737 mmHg; environmental temperature, 23.4–29 C); rhinoceros rectal body temperature, 35.8–37.5 C. The dashed line indicates the time at which butorphanol was administered. The asterisk (*) indicates a significant ($P< 0.05$) difference within treatment between t = 0 and t = 10. The pound symbol (#) indicates a significant ($P <0.05$) difference within treatment between t = 10 and t = 25. The infinity symbol (∞) indicates a significant ($P<0.05$) difference with treatment between t = 0 and t = 25.

TABLE 2. Distribution of arterial blood gases collected from the auricular artery and respiratory parameters,[a] with median and interquartile range (25th–75th percentile), at sampling periods 0, 5, 10, and 25 min in six 5- to 6-yr-old captive male white rhinoceros (*Ceratotherium simum*) for two treatments: etorphine-azaperone (EA) intramuscularly (IM) and etorphine-azaperone IM plus butorphanol intravenously (EAB IV). The study was conducted at Skukuza, Kruger National Park, South Africa (elevation, 281 m; atmospheric pressure, 734–737 mmHg; environmental temperature, 23.4–29 C); rhinoceros rectal body temperature, 35.8–37.5 C. The table includes two additional treatments, etorphine IM (E) and etorphine IM plus butorphanol IV (EB), administered as part of the same multivariant crossover study, as reported previously (Buss et al. 2018).

Treatment	0 min	5 min	10 min	25 min
PaO_2 (mmHg)				
5EA	24 (21–27)	26 (24–28)	27 (25–29)	26 (23–2.8)
EAB	24 (20–26)	49 (43–49)	46 (45–48)	39 (36–40)
E	25 (23–28)	25 (23–28)	28 (23–29)	26 (25–29)
EB	26 (22–26)	48 (42–50)	49 (42–51)	44 (38–46)
$P(A\text{-}a)O_2$ (mmHg)				
EA	53 (47–61)	45 (44–47)	46 (42–49)	41 (36–49)
EAB	44 (42–48)	39 (34–50)	40 (38–43)	46 (44–47)
E	42 (37–45)	49 (39–53)	44 (34–47)	39 (34–44)
EB	37 (33–42)	39 (33–43)	37 (31–39)	36 (35–39)
$PaCO_2$ (mmHg)				
EA	65 (62–72)	74 (72–78)	72 (67–75)	74 (70–81)
EAB	79 (77–80)	59 (54–62)	59 (52–61)	60 (57–65)
E	76 (67–81)	71 (61–80)	72 (67–82)	79 (71–82)
EB	81 (76–89)	58 (55–68)	63 (58–75)	64 (63–65)
$\dot{V}E_{BTPS}$ (L/min)				
EA	70 (68–79)	65 (61–69)	61 (51–71)	62 (55–67)
EAB	95 (94–105)	135 (109–140)	83 (73–87)	82 (76–89)
E	164 (127–182)	137 (103–154)	118 (89–131)	96 (67–101)
EB	151 (139–172)	153 (126–161)	90 (85–99)	83 (77–87)
fR (breaths/min)				
EA	4 (4–5)	4 (4–4)	4 (4–4)	6 (5–6)
EAB	5 (4–7)	10 (9–11)	6 (5–6)	7 (5–8)
E	10 (9–10)	9 (8–9)	7 (6–7)	6 (5–8)
EB	10 (8–10)	11 (10–13)	8 (6–8)	6.0 (6–7)
VT (L/breath)				
EA	15 (13–20)	14 (12–16)	16 (14–17)	11 (10–11)
EAB	17 (9–23)	12 (10–13)	12 (10–13)	11 (10–12)
E	18 (14–22)	16 (14–17)	18 (18–19)	14 (13–16)
EB	17 (15–20)	12 (12–16)	12 (11–17)	13 (12–14)
$\dot{V}D_{PHYS}$ (L/min)				
EA	23 (19–34)	26 (22–29)	23 (22–28)	24 (21–35)
EAB	33 (26–38)	40 (28–50)	29 (19–34)	24 (18–44)
E	60 (36–70)	42 (19–61)	40 (24–44)	36 (26–48)
EB	48 (47–51)	36 (18–52)	31 (22–41)	29 (26–35)
$\dot{V}O_2$ (L/min)				
EA	4 (4–5)	4 (4–5)	4 (4–5)	3 (3–4)
EAB	6 (6–7)	5 (5–6)	3 (3–4)	4 (3–4)
E	11 (10–12)	9 (8–10)	8 (6–9)	7 (4–7)
EB	11 (9–12)	7 (6–8)	4 (4–5)	4 (4–5)

TABLE 2. Continued.

Treatment	0 min	5 min	10 min	25 min
$\dot{V}CO_2$ (L/min)				
EA	3 (3–4)	3 (3–3)	3 (2–4)	2 (2–3)
EAB	4 (4–5)	5 (4–5)	3 (3–3)	3 (3–3)
E	8 (8–11)	7 (6–8)	6 (5–7)	4 (4–5)
EB	9 (7–10)	7 (6–8)	4 (4–4)	4 (3–4)

[a] PaO_2 = arterial partial pressure of oxygen; P(A-a)O_2 = alveolar to arterial oxygen gradient; $PaCO_2$ = arterial partial pressure of carbon dioxide; $\dot{V}E_{BTPS}$ = expired minute ventilation; $\dot{V}D_{PHYS}$ = physiologic dead space; fR = respiratory rate; VT = tidal volume; $\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbon dioxide production.

receiving butorphanol (Buss et al. 2018), in our study, changes in PaO_2 and $PaCO_2$ after butorphanol administration appeared to also be influenced by differences in oxygen and carbon dioxide solubility. Median $PaCO_2$ was not correlated with changes in $\dot{V}CO_2$ after butorphanol administration. This result implies that $PaCO_2$ was not only influenced by metabolic activity and carbon dioxide production but also by elimination in the lungs. Although pulmonary exchange of oxygen and carbon dioxide are similarly affected by ventilation and perfusion perturbations, the increased water solubility of carbon dioxide compared with that of oxygen supports a hypothesis that the difference in elimination of the two gases resulted in a loss

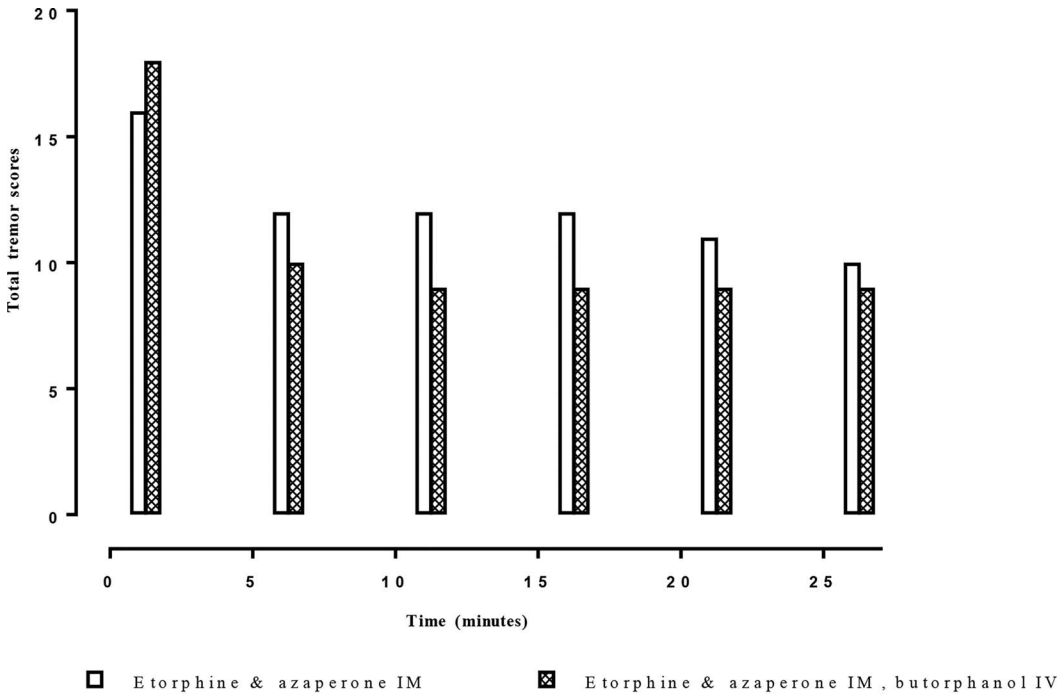

FIGURE 4. Muscle tremor scores at sampling periods 0, 5, 10, 15, 20, and 25 min were the sum of all the scores (1–5) at each time point in six 5- to 6-yr-old captive male white rhinoceros (*Ceratotherium simum*) for treatment etorphine plus azaperone (intramuscularly [IM]) or treatment etorphine plus azaperone (IM) and butorphanol intravenously (IV). Muscle tremor scores were subjectively evaluated by a single observer, with 1 indicating no visible tremors and 5 indicating severe tremors. The study was conducted at Skukuza, Kruger National Park, South Africa (elevation, 281 m; atmospheric pressure, 734–737 mmHg; environmental temperature, 23.4–29 C); rhinoceros rectal body temperature, 35.8–37.5 C.

TABLE 3. Overall distribution of blood gases collected from the auricular artery and respiratory parameters,[a] with median and interquartile range (25th–75th percentile), at sampling periods 0, 5, 10, and 25 min in six 5- to 6-yr-old captive male white rhinoceros (*Ceratotherium simum*) for two treatments: etorphine-azaperone intramuscularly (EA) and etorphine-azaperone intramuscularly plus butorphanol intravenously (EAB). The study was conducted at Skukuza, Kruger National Park, South Africa (elevation, 281 m; atmospheric pressure, 734–737 mmHg; environmental temperature, 23.4–29 C); rhinoceros rectal body temperature, 35.8–37.5 C.

	EA	EAB
PaO_2 (mmHg)	25 (23–28)	42 (40–46)
$P(A\text{-}a)O_2$ (mmHg)	45 (38–50)	42 (38–47)
$PaCO_2$ (mmHg)	73 (68–8)	60 (55–62)
$\dot{V}E_{BTPS}$ (L/min)	66 (56–70)	78 (73–90)
f_R (breaths/min)	4 (4–5)	6 (5–8)
VT (L/breath)	14 (11–17)	11 (9–13)
D_{PHYS} (L/min)	25 (21–33)	27 (19–38)
$\dot{V}O_2$ (L/min)	3.7 (3.1–4)	3.5 (3–3.9)
$\dot{V}CO_2$ (L/min)	2.7 (2.2–3.3)	2.9 (2.4–3.2)

[a] PaO_2 = arterial partial pressure oxygen; $P(A\text{-}a)O_2$ = alveolar to arterial oxygen gradient; $PaCO_2$ = partial pressure of carbon dioxide; $\dot{V}E_{BTPS}$ = expired minute ventilation; $\dot{V}D_{PHYS}$ = physiologic dead space; fR = respiratory rate; VT = tidal volume; $\dot{V}O_2$ = oxygen consumption; $\dot{V}CO_2$ = carbon dioxide production.

of association between $\dot{V}CO_2$ and $PaCO_2$ (Powers and Dhamoon 2020). Because the association between $\dot{V}CO_2$ and $PaCO_2$ persisted in etorphine-butorphanol rhinoceros, these findings suggest that the different outcome in our study is related to concurrent administration of azaperone with etorphine (Buss et al. 2018).

The addition of azaperone to etorphine in this study resulted in initial reduced muscle trembling and limb movement, which was associated with lower $\dot{V}O_2$ and $\dot{V}CO_2$ than those of animals receiving etorphine alone (Buss et al. 2018). Etorphine-immobilized white rhinoceros are reported to have increased metabolic activity (measured by $\dot{V}O_2$ and $\dot{V}CO_2$) that was associated with the degree of limb movements and muscle trembling (Buss et al. 2018). The decreased tremor scores suggest azaperone may reduce metabolic activity, thereby impacting oxygen consumption and carbon dioxide production. Because azaperone is a dopamine receptor antagonist, it may influence the activity of dopamine, which plays a role in a variety of central and peripheral metabolic processes (Leysen and Gommeren 2008; Rubí and Maechler 2010).

The findings of our study at t = 0 also suggest that etorphine-azaperone–immobilized rhinoceros had reduced ventilation compared with that of etorphine-immobilized rhinoceros. In etorphine-azaperone–immobilized rhinoceros, median $\dot{V}E_{BTPS}$ at the start of data recording was lower than that of $\dot{V}_{EXP}$ and $\dot{V}E_{BTPS}$ reported for rhinoceros receiving etorphine (Buss et al. 2018). The overall median $\dot{V}E_{BTPS}$ was also lower in the study rhinoceros administered etorphine-azaperone compared with etorphine alone (Buss et al. 2018). Because there were limited differences in blood gas values between etorphine-azaperone– versus etorphine-immobilized rhinoceros, ventilation may have been reduced in response to the decreased metabolic oxygen requirements and carbon dioxide produced (Buss et al. 2018).

The reduced $\dot{V}E_{BTPS}$ in etorphine-azaperone–treated rhinoceros was probably related to the decreased $\dot{V}CO_2$, compared with etorphine alone (Buss et al. 2018); however, a direct suppression of ventilation by azaperone cannot be excluded. The suppression of respiratory central and peripheral chemoreceptors and inhibition of ventilation by opioids are well documented (McDonald and Lambert 2005; Pattinson 2008); however, the contribution of azaperone to etorphine-associated ventilatory depression is unknown and requires further investigation (Kolesnikova and Serebrovskaya 1998). Azaperone may influence ventilation through its antagonistic action at multiple receptors that are believed to influence breathing, including dopamine-, $alpha_1$-, $histamine_1$-, 5-hydroxytyptamine-, and $muscarinic_3$-receptors (Lemke 2007; Leysen and Gommeren 2008; Burroughs et al. 2012).

Our results suggest that etorphine-azaperone causes an elevated $P(A\text{-}a)O_2$ (median, 53 mmHg). The normal gradient in white rhinoceros at rest is unknown; however, when comparing results in etorphine-azaperone–immobilized rhinoceros with those in horses at rest (10 mmHg; Doherty

and Valverdis 2008), it suggests an increase of clinical significance in rhinoceros. Similar increases in $P(A\text{-}a)O_2$ have been reported for rhinoceros immobilized with etorphine only (Buss et al. 2018). These results suggest that azaperone has limited effect on the A-a gradient changes induced by etorphine. Etorphine markedly increased pulmonary arterial pressure in immobilized white rhinoceros, and gas exchange across alveolar-capillary membranes may be reduced by pulmonary congestion, interstitial oedema, increased speed of blood flow through pulmonary vasculature, or ventilation-perfusion mismatching (Meyer et al. 2015; Boesch et al. 2018; Mosing et al. 2020). The inclusion of azaperone probably reduces etorphine-associated systemic arterial hypertension by blocking $alpha_1$-receptors at peripheral arterioles (Buss et al. 2016); however, the effects of azaperone on pulmonary vasculature and blood pressure within the lungs are unknown. Studies on how azaperone might influence lung perfusion, ventilation, or both are still required (Lemke 2007).

In this study, the small sample size (six rhinoceros) may have reduced statistical power and masked additional physiologic changes of clinical importance. In addition, a more complete understanding of the physiologic mechanisms influencing blood gases was limited by an inability to assess alveolar ventilation, cardiac output, pulmonary artery pressures, shunt fractions, and ventilation:perfusion ratios. Differences in physiologic responses may exist between captive and free-ranging rhinoceros, and further studies should compare these conditions.

Overall, our study demonstrated a clinical benefit of butorphanol administration in etorphine-azaperone–immobilized white rhinoceros, with decreases in both hypoxia and hypercapnia. This effect appeared to be associated with reduced metabolic activity. Although the arterial partial pressures of oxygen and carbon dioxide measured in white rhinoceros immobilized with etorphine-azaperone were not clinically different from values in white rhinoceros that received only etorphine, based on the metabolic-sparing effects of azaperone, we recommend that azaperone should be included with etorphine in the chemical immobilization of white rhinoceros.

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