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ORIGINAL RESEARCH

Investigation of cardiorespiratory effects of the selective 5-HT4 agonist BIMU-8 in etorphine-immobilised goats (*Capra aegagrus hircus*) in a randomized, blinded and controlled trial

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Abstract

Background: Opioid-induced respiratory compromise remains a significant challenge in etorphine-immobilised wildlife. Serotonergic agonists offer a potential avenue for preventing or treating opioid-induced respiratory compromise. We therefore aimed to determine whether the selective 5-hydroxytryptamine receptor 4 (5-HT4) agonist, BIMU-8, reverses opioid-induced respiratory compromise in etorphine-immobilised goats.

Methods: Seven healthy adult goats were immobilised with etorphine, then treated with BIMU-8 or sterile water 5 minutes later in a randomised, prospective cross-over study. Cardiorespiratory variables were measured at 1-minute intervals from 4 minutes before etorphine to 15 minutes after its administration. Arterial blood gas analyses were also performed before and after etorphine administration and the respective treatments.

Results: Intravenous injection of BIMU-8 attenuated etorphine-induced respiratory compromise, as indicated by improvements, compared to baseline and between treatments, in respiratory rate (f_R), peripheral arterial blood oxygen saturation (SpO_2), partial pressure of arterial oxygen (PaO_2) and the alveolar-arterial oxygen partial pressure gradient ($P(A\text{-}a)O_2$). BIMU-8 caused an increase in heart rate and a temporary decrease in arterial blood pressure. Mild movements and slight muscle spasm occurred but BIMU-8 did not reverse immobilisation.

Conclusion: Our results indicate that BIMU-8 may be a potential drug candidate for the treatment, or prevention, of etorphine-induced respiratory compromise in immobilised ungulates.

KEYWORDS
BIMU-8, boer goat, etorphine, opioid-induced respiratory depression

INTRODUCTION

Chemical immobilisation is an essential tool for the management, conservation and veterinary care of captive and free-ranging wildlife species. The potent opioid etorphine is routinely used for the chemical capture of large herbivores as a small volume reliably induces a rapid and reversible immobilisation. However, together with etorphine's desirable effects, well recognised opioid-induced respiratory compromise remains a major concern.[1]

The functional relationship between opioids and respiration is difficult to untangle, but it is generally accepted that opioid-induced respiratory compromise is primarily mediated via μ-opioid receptors. Mu-opioid receptors are abundantly expressed in the Pre-Bötzinger Complex (PBC), an area of the brainstem responsible for respiratory rhythm generation in mammals.[2–6] Activation of μ-opioid receptors decreases inspiratory drive within the PBC[4,7] and supresses the normal hypoxic or hypercapnic respiratory drive, which is triggered via peripheral and central chemoreceptors.[3,8,9] Apart from the centrally-mediated respiratory effects of opioids, recent evidence suggests that the administration of potent μ-opioids, such as etorphine, leads to pulmonary

hypertension in herbivores such as goats and rhinoceros which hinders gas exchange.[10,11] Together, these opioid-induced effects on respiration may lead to potentially lethal hypercapnia and hypoxaemia.[3]

The respiratory neurones in the PBC depend on neurotransmitters such as serotonin (5-hydroxytryptamine or 5-HT) for the generation of respiratory rhythm.[2] Serotonin modulates respiratory rhythm generation through its actions on 5-HT1A, 5-HT4 and 5-HT7 serotonin receptors in mammals.[12,13] Both μ-opioid receptors and 5-HT4 receptors are co-expressed on respiratory neurones in the PBC, but display opposing actions.[14]

Manzke et al[7] confirmed that the 5-HT4 agonist, BIMU-8, reverses fentanyl-induced respiratory depression and apnoea, without affecting analgesia in rats. More relevant to herbivores is the finding that zacopride, another 5-HT4 agonist with 5-HT3 antagonistic effects, was found to alleviate opioid-induced respiratory depression in goats, when co-administered with etorphine.[15] Although zacopride did not significantly affect catatonic immobilisation in goats, some loss of sedation, attributed to zacopride's 5-HT3 antagonistic effects, was noted.[15]

BIMU-8 is more selective than zacopride for the 5-HT4 receptor (BIMU-8 is a selective 5-HT_{4alpha} receptor agonist) thus sedation and catatonic immobilisation may be better maintained when BIMU-8, rather than zacopride, is used to reverse respiratory compromise in etorphine-immobilised herbivores.

The aim of this study was to evaluate the effect of BIMU-8 on respiratory compromise in etorphine-immobilised goats. We hypothesised that BIMU-8 would attenuate etorphine-induced respiratory compromise while maintaining immobilisation. We used domestic goats (*Capra aegagrus hircus*) as a model species to identify the lowest effective dose of BIMU-8 and subsequently evaluate its effects on an ungulate's cardiovascular and respiratory system without the confounding effects of a severe stress response that normally accompanies wildlife capture.

MATERIALS AND METHODS

Animals

All procedures were approved by the Animal Ethics Committee of the University of Pretoria (clearance V031-18).

Seven healthy, adult, female Boer goats with a mean body mass of 31.5 kg (standard deviation: ±5.5 kg) were used. The goats were housed at the Biomedical Research Centre of the Onderstepoort Veterinary Faculty, South Africa, at an altitude of 1753 m. The goats had access to water and hay *ad libitum* and were fed supplementary sheep concentrate pellets and lucerne. Over a period of 3 weeks, before the start of the experiments, the goats were habituated to a face mask and sling restraint.

EXPERIMENTAL PROCEDURES

Experimental set up

The goats were weighed before each trial and were starved for 16 hours before anaesthesia to reduce the risk of bloat and regurgitation. The animals were restrained in a sternal position in a sling with their heads held upright. A canine anaesthetic face mask (J-298C, Jorgensen Laboratories, CO, USA) was placed over the nose and mouth of the goat and sealed tightly with an adapted latex glove. The mask was attached to a two-way valve (2730 Series; Hans Rudolph, Inc., OK, USA) which led all expired air through a gas mixing chamber (MLA245) and respiratory flow head (MLT1000L) connected via a spirometer (ML140) to a PowerLab Exercise Physiology System (ML870B80, ADInstruments, NSW, Australia). The Metabolic Module within the LabChart software (Chart 5, ADInstruments Pty Ltd, NSW, Australia) was used to measure and calculate respiratory rate (f_R), minute ventilation ($\dot{V}_E$) and tidal volume (V_T). The spirometer was zeroed, and a two-point calibration was performed using no air flow and 3 L of air, using a 3 L calibration syringe, before each set of measurements.

Peripheral blood haemoglobin oxygen saturation (SpO_2) was measured using pulse oximetry (PalmSat, Nonin Medical Inc., MN, USA), with a transflectance probe attached to the hairless ventral aspect of the goat's tail.

The ears and right lateral neck were shaved and swabbed with disinfectant (F10 SC, Health and Hygiene (Pty) Ltd., GT, South Africa) before lidocaine cream (Emla 5%, AstraZeneca Pharmaceuticals (Pty) Ltd., DE, USA) was applied to these areas. An 18-gauge IV catheter (Jelco, Smiths Medical International Ltd., KNT, UK) was placed in the right jugular vein to facilitate intravenous administration of BIMU-8 (magistral preparation, BOKU, Austria), sterile water and diprenorphine hydrochloride (M5050 12 mg ml^{-1}, Novartis South Africa (Pty) Ltd/(Edms) Bpk, GT, South Africa). A 22-gauge IV catheter (Jelco, Smiths Medical International Ltd., KNT, UK) was placed in the auricular artery of one ear for blood gas sampling, and another was placed in the auricular artery of the opposite ear and connected via a fluid-filled arterial line to a manufacturer pre-calibrated (-50 – 300 mm Hg) Deltran II pressure transducer (DPT-200, Utah Medical Products, UT, USA.). The transducer was secured at the level of the scapulohumeral joint (level of the heart base) and was zeroed to atmospheric pressure before each experiment.

The transducer was connected via a blood pressure amplifier (FE117, ADInstruments, NSW, Australia) to the PowerLab System, which assessed real-time data of direct mean (MAP), systolic (SAP) and diastolic (DAP) arterial blood pressures as well as heart rate (HR) using the LabChart software.

Body temperature (BT) was measured using a rectally placed temperature probe (Checktemp Electronic Thermometer, Hanna Instruments, RI, USA).

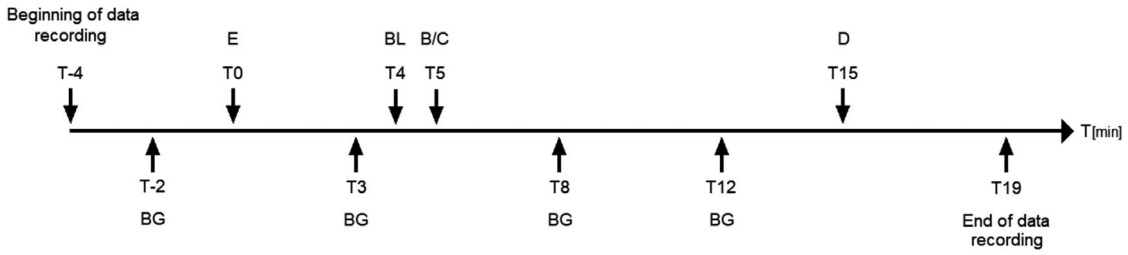

FIGURE 1 **Experimental timeline**. T: timepoint; BG: sampling of arterial blood for blood gas analysis (T-2, T3, T8, T12); E: administration of 0.1 mg kg^{-1} etorphine hydrochloride IM (T0); BL = baseline measurements (T4); B/C = administration of 3 mg kg^{-1} BIMU-8 (B) or sterile water equal in volume to the amount of BIMU-8 in mL (control [C]) IV; (T5); D = administration of diprenorphine (T15)

Electrocardiogram (ECG) was recorded by a Bio Amp amplifier (ML132, BIO Amp CF), which also interfaced with the PowerLab System. ECG pads (200 Foam Electrodes + Conductive Adhesive Hydrogel, Covidien (Kendall) llc, MA, USA) were attached to shaved areas on the goat's left hind leg, right and left front legs, about 5 centimetres dorsal to the tarsal and carpal joint, respectively.

Arterial blood samples were drawn with a heparinised 1 mL syringe (B. Braun, Melsungen AG, HE, Germany). The arterial catheter was flushed with 2 mL heparinised saline (5 i.U. mL^{-1} Heparin Sodium, Fresenius Kabi Manufacturing SA (Pty) Ltd, EC, South Africa) after each sample collection. The arterial samples were sealed, stored on ice and analysed within 3 minutes. A portable blood gas analyser (EPOC Blood Analysis System, Epocal, ON, Canada) with precalibrated blood gas cassettes (BGEM smart cards, Epocal, ON, Canada) was used to measure arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$), and pH, corrected for the animal's BT.

All measurements were taken indoors between 9 AM and 3 PM (GMT +2) at an ambient dry-bulb temperature of 21°C. The barometric pressure, measured by the on-board barometer of the blood gas analyser, ranged from 657.0 to 665.2 mm Hg (87.6–88.7 kPa). Therefore, the partial pressure of inspired oxygen was about 139 mm Hg (18.5 kPa) at the given altitude.

Experimental procedures

The study was a randomised controlled prospective crossover study where the primary investigators were blinded to the treatments. Animals were randomly assigned using a random sequence generator (random.org) and received the following two interventions: etorphine + BIMU-8 (treatment) and etorphine + sterile water (control), with a wash-out period of 7 days between interventions. The effective dose of BIMU-8 (3 mg kg^{-1}) was determined in a pilot dose-response study. Power analysis based on data obtained in the pilot study indicated that seven goats would permit the detection of a 10% difference in PaO_2 with a standard deviation (SD) of 4 mm Hg between BIMU-8 and control treatment, with an alpha level of 0.05 and a power of 0.90.

All animals were immobilised with 0.1 mg kg^{-1} etorphine hydrochloride (M99, 9,8 mg ml^{-1}, Novartis South Africa (Pty) Ltd, GT, South Africa) injected intramuscularly (IM) into the lateral gluteal muscle. The variables f_R, SpO_2, $\dot{V}_E$, V_T, HR, SAP, DAP, MAP and BT, as well as movement score and palpebral reflex were assessed in 1-minute intervals starting 4 minutes (T-4) before and ending 19 minutes (T19) after etorphine (T0) injection (see experimental timeline Figure 1). ECG was recorded continuously. BIMU-8 (CAS Number 134296-40-5) (3 mg kg^{-1}) or sterile water was injected intravenously (IV) over a 30 second duration into the right jugular vein, 5 minutes after etorphine (T5). Arterial blood gas samples were taken at 2 minutes (T-2) before and 3 (T3), 8 (T8) and 12 (T12) minutes after etorphine injection.

TABLE 1 Index used to describe the level of immobilisation in goats

Score	Description
0	No visible movement of any part of the body
1	slight, unintentional movement or spasms of the head or legs; palpebral reflex absent
2	In addition to score 1, slight movement of the lips, ears or tail
3	Minimal purposeful, non-rhythmic movement of the head or legs
4	Moderate to strong spasms of the hind legs and pronounced tail movement
5	Pronounced deliberate movement of either the legs or the head
6	Strong deliberate movement of the whole body

The level of immobilisation was assessed using a movement score (Table 1). Additionally, depth of anaesthesia was monitored by evaluating palpebral reflex and magnitude of miosis.

Immobilisation was reversed 15 minutes (T15) after etorphine injection by administration of 0.2 mg kg^{-1} diprenorphine hydrochloride administered IV. At the end of the recording period, 4 minutes after reversal (T19), all instruments were removed, and the goats were returned to their stables.

Data analysis

Statistical analyses were performed with R Studio (R version 3.4.1[16]).

Differences between BIMU-8 and control were assessed using linear mixed effects models (lme4 package,[17]). A residual plot and a histogram of the residual were performed to check for homoscedasticity and normal distribution in the data. A post hoc

test (Tukey test) (lsmeans package,[18]) for multiple comparisons with p-value adjustment tested differences in the measured variables between BIMU-8 and control over time; and between baseline values (T4) and selected time points within treatments. Data of all animals were included in the analysis, except for incoherent SpO_2-data of one animal that was excluded due to problems with contact of the pulse oximeter probe.

The partial pressure of oxygen in the pulmonary alveoli (PAO_2) was calculated using the Alveolar Gas Equation (FiO_2*(Pb-PH_2O)-$PaCO_2$/RQ), where the fractional inspired oxygen (FiO_2) was determined as 0.21 (percentage of oxygen in atmospheric air), and the measured barometric pressure (Pb) was 661.78 mm Hg. The partial pressure of water vapour in the alveoli (PH_2O) was calculated for the given BT of each animal at the respective time-points. A respiratory quotient (RQ) of 1 was used as previously reported for healthy Boer goats.[19] We evaluated the alveolar-arterial oxygen partial pressure gradient ($P(A\text{-}a)O_2$) by calculating PAO_2–PaO_2.

Non-normally distributed movement score data were assessed with an ordinal logistic regression method. A cross table was created to compare the incidence of BIMU-8 and control for each grade of the movement score.

Data are reported as mean (standard error of the mean [SEM]). A p-value of < 0.05 was considered statistically significant.

RESULTS

Respiratory rate

All animals showed pronounced hypopnea after etorphine administration with a decrease of f_R from 27 (±4) breaths min^{-1} to 15 (±4) breaths min^{-1} ($p = 0.001$) in the BIMU-8 treatment and from 30 (±4) breaths min^{-1} to 20 (±4) breaths min^{-1} ($p = 0.0081$) in the control treatment 2 minutes pre-etorphine to 4 minutes post-etorphine (Figure 2a, Table 2). The administration of BIMU-8 resulted in a gradual increase of f_R which differed from the control treatment ($p < 0.03$) 6–9 minutes post-BIMU-8/control and baseline ($p < 0.05$) 3–14 minutes post-BIMU-8/control (except T9) (Figure 2a, Table 2).

Peripheral blood oxygen haemoglobin saturation

Etorphine administration resulted in a decrease of SpO_2 from 96 (±3)% to 84 (±3)% in the BIMU-8 treatment ($p = 0.0003$) and from 92 (±3)% to 83 (±3) % in the control treatment ($p = 0.005$) 2 minutes pre-etorphine to 4 minutes post-etorphine (Figure 2b, Table 2). BIMU-8 increased SpO_2 to 91 (± 3)% compared to 85 (±3)% in the control treatment ($p < 0.05$) 2 minutes post-BIMU-8/control and remained elevated until 7 minutes post-BIMU-8/control (except T9) (Table 2).

Arterial blood gases

After etorphine, PaO_2 decreased from 77.1 (±3.3) mm Hg to 62.7 (±3.3) mm Hg ($p = 0.0095$) in the BIMU-8 treatment and from 79.5 (±3.3) mm Hg to 62.1 (±3.3) mm Hg ($p = 0.0013$) in the control treatment 2 minutes pre-etorphine to 3 minutes post-etorphine (Figure 3a, Table 3).

Administration of BIMU-8 led to an increase in PaO_2 (78.8 [±3.3] mm Hg at T12), which significantly differed from 3 minutes post-etorphine ($p = 0.0032$) 7 minutes post-BIMU-8/control and the control until the end of anaesthesia ($p = 0.0231$, 3 minutes post-BIMU-8/control; $p = 0.0062$, 7 minutes post-BIMU-8/control) (Table 3).

Although $PaCO_2$ (Figure 3b) tended to increase in all animals after etorphine, this change was not statistically significant (Table 3, Figure 3b).

BIMU-8 led to a decrease in $PaCO_2$ compared to the control treatment (BIMU-8: 43.0 [±1.9] mm Hg, control: 49.5 [±1.9] mm Hg, $p = 0.0098$, 7 minutes post-BIMU-8/control) (Table 3).

Following etorphine $P(A\text{-}a)O_2$ increased significantly (BIMU-8: 10.6 [±2.7] mm Hg to 19.6 [±2.7] mm Hg, $p = 0.021$; control: 4.9 [±2.7] mm Hg to 19.1 [±2.7] mm Hg, $p < 0.001$) 2 minutes pre-etorphine to 3 minutes post-etorphine (Figure 3c, Table 3). $P(A\text{-}a)O_2$ was significantly lower after treatment with BIMU-8 compared to control (BIMU-8: 12.8 [±2.7] mm Hg, control: 18.9 [±2.7] mm Hg, $p = 0.0436$, 3 minutes post-BIMU-8/control) and compared to 3 minutes post-etorphine ($p < 0.001$, 19.6 (±2.7) mm Hg versus 6.3 [±2.7] mm Hg) (Table 3).

Other respiratory variables

Minute ventilation ($\dot{V}_E$) decreased clinically but not significantly after etorphine (Table 2). BIMU-8 administration led to a gradual increase in $\dot{V}_E$, which became significant 6 minutes post-BIMU-8/control compared to the control (BIMU-8: 7.7 [±1.3] L min^{-1}; control: 5.0 [±1.3] L min^{-1}; $p = 0.0216$). $\dot{V}_E$ in the BIMU-8 group then remained elevated from 6 minutes post-BIMU-8/control until antagonism of etorphine (Table 2). After diprenorphine administration, $\dot{V}_E$ increased in the BIMU-8 treatment ($p < 0.05$) to a maximum of 10.7 (±1.3) L min^{-1} at T15 and in the control treatment ($\dot{V}_E$ = 10.0 [±1.3] L min^{-1}, T18, $p < 0.05$) compared to baseline.

Tidal volume did not significantly differ between treatments or baseline throughout the immobilisation (Table 2).

Cardiovascular variables

Following etorphine, HR did not change significantly in the BIMU-8 treatment from 75 (±7) beats min^{-1} to 68 (±7) beats min^{-1} or in the control treatment from 80 (±7) beats min^{-1} to 72 (±7) beats min^{-1} 2 minutes pre-etorphine to 4 minutes post-etorphine (Figure 4a,

TABLE 2 Mean ± standard error of respiratory rate (f_R), peripheral arterial blood oxygen saturation (SpO_2) (n=6), minute ventilation ($\dot{V}_E$), tidal volume (V_T), heart rate (HR), mean arterial pressure (MAP) and rectal temperature (RT) in Boer goats (n = 7) measured before (T-4 – T-2) and after (T2 – T4) administration of 0.1 mg kg^{-1} etorphine hydrochloride IM (at T0); at baseline (T4) and after (T5 - T15) administration of 3 mg kg^{-1} BIMU-8 or sterile water equal amount of mL BIMU-8 (control [C]) IV at T5. Etorphine was antagonised with diprenorphine IM at T15. Gray shaded timepoints indicate: Administration of etorphine (T0), baseline (T4), administration of BIMU-8/control treatment (T5) and administration of diprenorphine (T15)

		Pre-etorphine		**Etorphine**		**BIMU-8/Control**										**Diprenorphine**		
		Time (minutes)																
Variable	**Treatment**	**−4**	**−2**	**0**	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**17**	**19**
f_R (breaths min^{-1})	Control	31 (4)	30 (4)	29 (4)	24 (4)	20 (4)°	18 (4)°	18 (4)°	19 (4)°	18 (4)°	18 (4)°	18 (4)°	17 (4)°	17 (4)°	17 (4)°	17 (4)°	25 (4)	29 (4)*	27 (4)
	BIMU-8	27 (4)	27 (4)	28 (4)	27 (4)	15 (4)°	17 (4)°	19 (4)°	18 (4)°	23 (4)*	22 (4)	23 (4)*	27 (4)*,†	26 (4)*,†	26 (4)*,†	26 (4)*,†	31 (4)*	32 (4)*	29 (4)*
SpO_2 (%)	Control	92 (3)	92 (3)	93 (3)	87 (3)	83 (3)°	84 (3)°	85 (3)°	85 (3)°	86 (3)	88 (3)	86 (3)	86 (3)	87 (3)	88 (3)	89 (3)	89 (3)	90 (3)*	91 (3)*
	BIMU-8	95 (3)	96 (3)	94 (3)	92 (3)	84 (3)°	86 (3)°	87 (3)°	91 (3)*,†	93 (3)*,†	93 (3)*	93 (3)*,†	95 (3)*,†	95 (3)*,†	90 (3)°	90 (3)°	91 (3)*	88 (3)°	88 (3)°
$\dot{V}_E$ ($L\ min^{-1}$)	Control	8.2 (1.3)	8.0 (1.3)	7.8 (1.3)	6.3 (1.3)	5.6 (1.3)	5.3 (1.3)	5.1 (1.3)	5.3 (1.3)	5.1 (1.3)	5.0 (1.3)	5.1 (1.3)	5.0 (1.3)	5.1 (1.3)	4.9 (1.3)	5.1 (1.3)	9.6 (1.3)	9.0 (1.3)	11.7 (1.3)
	BIMU-8	6.9 (1.3)	6.9 (1.3)	7.1 (1.3)	6.8 (1.3)	4.7 (1.3)	4.8 (1.3)	6.0 (1.3)	6.7 (1.3)	7.0 (1.3)	6.3 (1.3)	6.3 (1.3)	7.7 (1.3)†	7.4 (1.3)	7.4 (1.3)†	8.3 (1.3)†	10.7 (1.3)*	9.7 (1.3)*	7.3 (1.3)
V_T (L)	Control	0.26 (0.03)	0.26 (0.03)	0.24 (0.03)	0.24 (0.03)	0.27 (0.03)	0.26 (0.03)	0.25 (0.03)	0.27 (0.03)	0.27 (0.03)	0.28 (0.03)	0.28 (0.03)	0.29 (0.03)	0.28 (0.03)	0.29 (0.03)	0.31 (0.03)	0.36 (0.03)°,*	0.31 (0.03)	0.32 (0.03)
	BIMU-8	0.24 (0.03)	0.24 (0.03)	0.25 (0.03)	0.25 (0.03)	0.30 (0.03)	0.28 (0.03)	0.27 (0.03)	0.29 (0.03)	0.25 (0.03)	0.24 (0.03)*	0.25 (0.03)	0.26 (0.03)	0.26 (0.03)	0.26 (0.03)	0.28 (0.03)	0.29 (0.03)	0.29 (0.03)	0.22 (0.03)*,†
HR (beats min^{-1})	Control	79 (7)	80 (7)	77 (7)	68 (7)	72 (7)	67 (8)	63 (7)	65 (7)	69 (7)	72 (7)	74 (7)	73 (7)	73 (7)	72 (7)	71 (7)	76 (7)	72 (7)	81 (7)
	BIMU-8	78 (7)	75 (7)	73 (7)	62 (7)	68 (7)	105 (8)°,*,†	118 (7)°,*,†	118 (7)°,*,†	118 (7)°,*,†	114 (7)°,*,†	111 (7)°,*,†	107 (7)°,*,†	104 (7)°,*,†	103 (7)°,*,†	108 (7)°,*,†	103 (7)°,*,†	113 (7)°,*,†	109 (7)°,*,†
MAP (mm Hg)	Control	90 (5)	88 (5)	96 (5)	109 (5)°	102 (5)°	106 (6)°	107 (5)°	103 (5)°	96 (5)	95 (5)	94 (5)	93 (5)	91 (5)	91 (5)	91 (5)	104 (5)°	104 (5)°	99 (5)°
	BIMU-8	94 (5)	92 (5)	94 (5)	111 (5)°	99 (5)	83 (5)*,†	90 (5)†	96 (5)	97 (5)	95 (5)	92 (5)	96 (5)	97 (5)	95 (5)	93 (5)	106 (5)°	105 (5)°	102 (5)
RT (^{0}C)	Control	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.0 (0.2)	39.0 (0.2)	39.0 (0.2)	39.0 (0.2)	38.9 (0.2)	38.9 (0.2)	38.9 (0.2)	38.9 (0.2)	38.8 (0.2)	38.7 (0.2)	38.5 (0.3)
	BIMU-8	39.0 (0.2)	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.1 (0.2)	39.0 (0.2)	39.0 (0.2)	38.9 (0.2)	38.9 (0.2)	38.9 (0.2)	38.8 (0.2)	38.8 (0.2)	38.8 (0.2)	38.7 (0.2)	38.8 (0.2)	38.7 (0.2)	38.7 (0.2)

° Significantly different from value at −2 minutes (2 minutes before etorphine administration (T-2)) (all $p < 0.05$).
* Significantly different from value 4 minutes after etorphine administration and 1 minute before injection of BIMU-8/control, (T4) ($p < 0.05$).
† Significantly different from control group at the same time point (all $p < 0.05$).

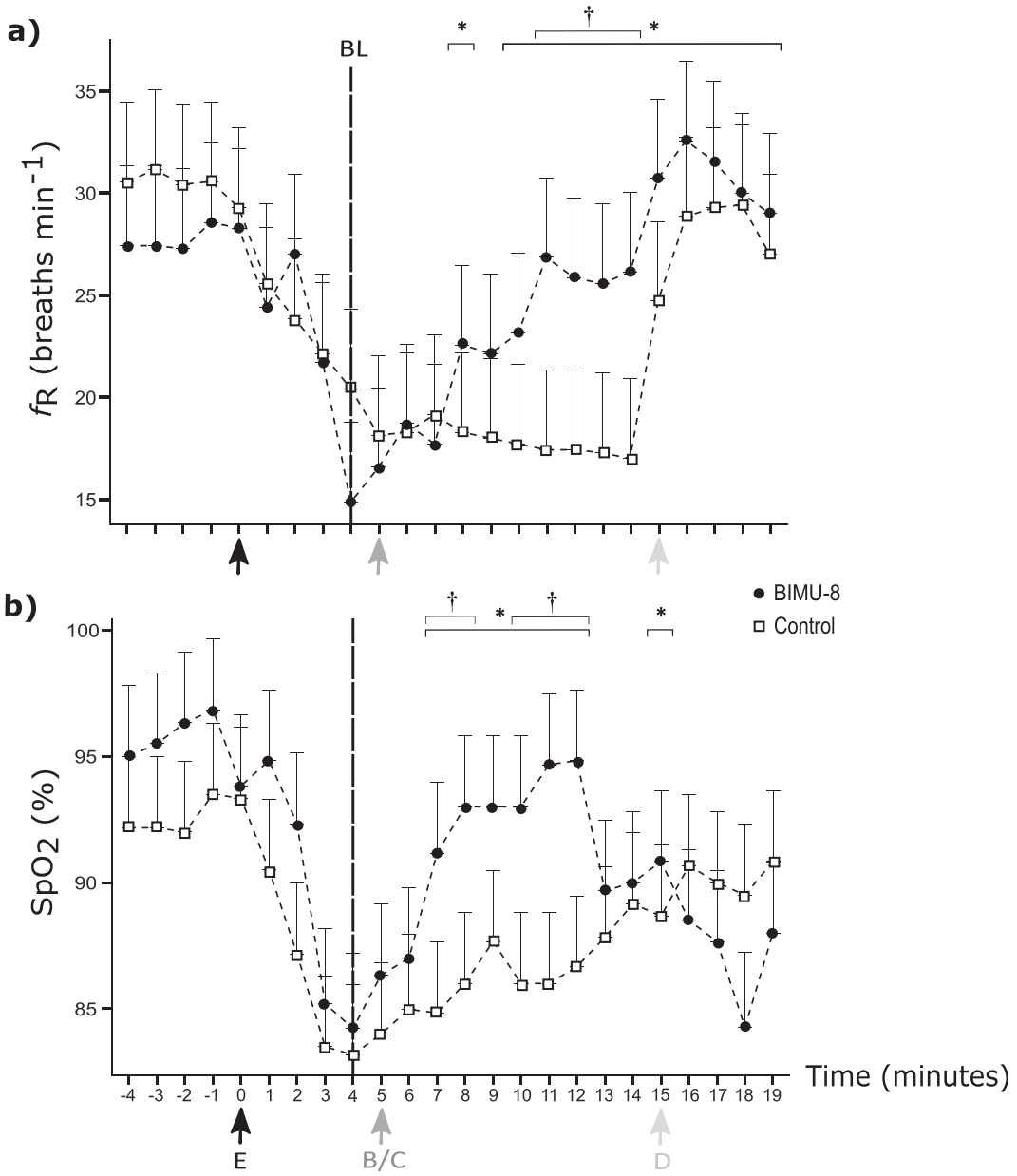

FIGURE 2 (**a and b**) Mean ± standard error of **(a)** respiratory rate (breaths min^{-1}) ($n = 7$) and **(b)** peripheral arterial oxygen haemoglobin saturation measured from pulse oximetry (%) ($n = 6$) in Boer goats administered 0.1 mg kg^{-1} etorphine hydrochloride IM and 3 mg kg^{-1} BIMU-8 or sterile water to the equal amount of mL BIMU-8 (control (C)) IV. †BIMU-8 treatment significantly different (all $p < 0.05$) from control at the respective time points. *BIMU-8 treatment (black dots) significantly different (all $p < 0.05$) from baseline (BL) at the respective time points. Arrows represent: E = etorphine injection (T0) (black arrow). BL = baseline (4 minutes after etorphine administration and 1 minute before injection of BIMU-8/control (T4) (dashed line)). B/C = administration of treatment (BIMU-8/control [T5] [dark grey arrow]). D = administration of diprenorphine, 15 minutes after etorphine administration (T15) (light grey arrow)

TABLE 3 Mean ± standard error of partial arterial oxygen pressure (PaO_2), partial arterial carbon dioxide pressure ($PaCO_2$) and alveolar-arterial oxygen partial pressure gradient ($P(A\text{-}a)O_2$) in Boer goats (n = 7) measured before (T-2) and after (T3) administration of 0.1 mg kg^{-1} etorphine hydrochloride IM; and after (T8 and T12) administration of 3 mg kg^{-1} BIMU-8 or sterile water equal amount of mL BIMU-8 (control (C)) IV

		Time point (minutes)			
Variable	**Treatment**	**−2**	**3**	**8**	**12**
$PaO_{2\ (mm\ Hg)}$	Control	79.5 (3.3)	62.1 (3.3)°	61.4 (3.3)°	66.3 (3.3)°
	BIMU-8	77.1 (3.3)	62.7 (3.3)°	71.6 (3.3)†	78.8 (3.3)*,†
$PaCO_{2\ (mm\ Hg)}$	Control	43.6 (1.9)	46.7 (1.9)	47.6 (1.9)	49.5 (1.9)
	BIMU-8	40.1 (1.9)	45.6 (1.9)	43.6 (1.9)	43.0 (1.9)†
$P(A\text{-}a)O_{2\ (mm\ Hg)}$	Control	4.9 (2.7)	19.1 (2.7)°	18.9 (2.7)°	12.2 (2.7)
	BIMU-8	10.6 (2.7)	19.6 (2.7)°	12.8 (2.7)†	6.3 (2.7)*

°Significantly different from value at −2 minutes (2 minutes before etorphine administration (T-2)) (all $p < 0.05$).
*Significantly different from value at 3 minutes (3 minutes after etorphine administration and 2 minutes before injection of BIMU-8/control (T3)) ($p < 0.05$).
†BIMU-8 treatment significantly different from value in the control group at the same time point (all $p < 0.05$).

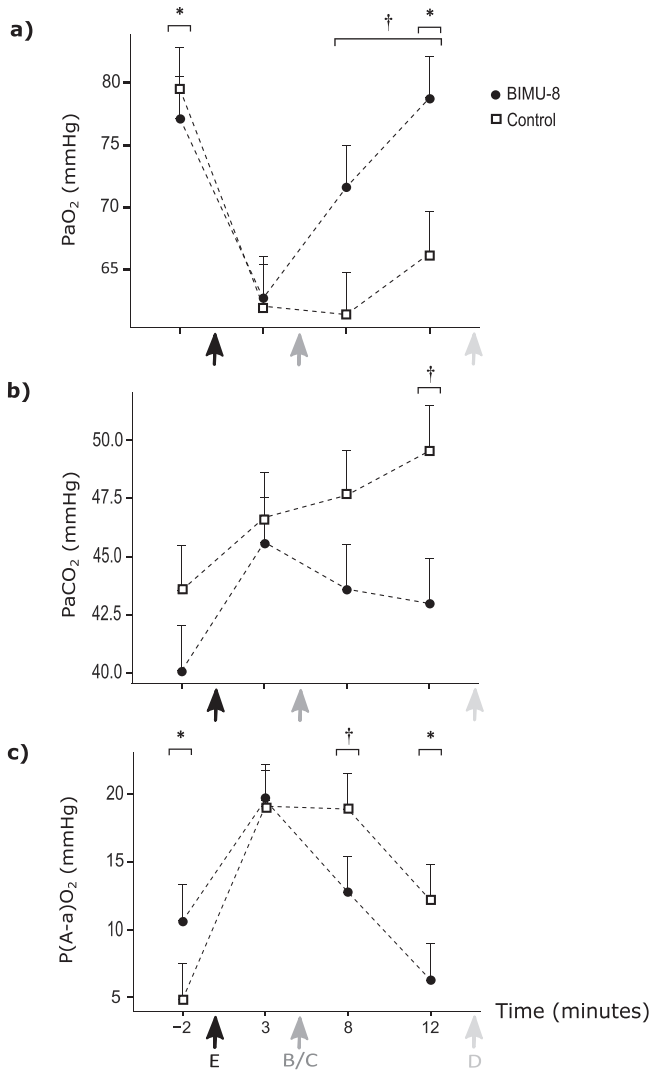

FIGURE 3 (**a-c**) Mean ± standard error of **(a)** partial pressure of oxygen in arterial blood (PaO_2 mm Hg) **(b)** partial pressure of carbon dioxide in arterial blood ($PaCO_2$ mm Hg) and **(c)** alveolar-arterial oxygen partial pressure gradient ($P(A\text{-}a)O_2$ mm Hg) in Boer goats ($n = 7$) measured before (T-2) and after (T3) administration of 0.1 mg kg^{-1} etorphine hydrochloride IM; and after (T8 and T12) administration of 3 mg kg^{-1} BIMU-8 or sterile water equal amount of mL BIMU-8 (control [C]) IV. †BIMU-8 treatment significantly different (all $p < 0.05$) from control at the respective time points. *BIMU-8 treatment (black dots) at T3 (after etorphine) significantly different ($p < 0.05$) from timepoint T-2 (pre-etorphine) and T8/ T12 (post BIMU-8 treatment). Arrows represent: E = etorphine administration (T0) (black arrow); B/C = administration of treatment (BIMU-8 or control) (T5) (dark grey arrow). D = administration of diprenorphine, 15 minutes after etorphine administration (T15) (light grey arrow)

Table 2). Administration of BIMU-8 led to an immediate and significant increase of HR from 68 (±7) beats min^{-1} to 118 (±7) beats min^{-1} and remained greater than the control ($p < 0.001$) and baseline ($p < 0.001$) until the end of data collection (Table 2).

Blood pressure increased after etorphine administration in all goats to a maximum MAP of 111 (±5) mm Hg in the BIMU-8 treatment ($p = 0.0012$) and 109 (±5) mm Hg in the control treatment ($p = 0.0004$) 2 minutes pre-etorphine to 2 minutes post-etorphine (Figure 4b, Table 2). BIMU-8 led to a rapid decrease in blood pressure to a minimum MAP of 83 (±5) mm Hg, which differed from baseline ($p = 0.0086$) and control ($p = 0.0003$) but returned to pre-etorphine levels within 2 minutes. From 3 minutes post-BIMU-8/control onwards, the goats' blood pressures were similar between the interventions (Table 2).

Movement

Administration of etorphine resulted in full immobilisation within 3 minutes after injection in all animals. The anaesthetic effects were characterised by the absence of vocalisation, reactions to external stimuli, palpebral reflex and a movement score of 0–4 minutes post-etorphine.

No changes were observed in the control treatment during the immobilisation, except for one animal. In this animal palpebral reflex diminished but did not fully disappear after etorphine and movement of the head, legs and tail occurred from 9 minutes post-etorphine onwards (score 5).

Administration of BIMU-8 caused slight movements and increased muscle stiffness in some of the animals: three goats developed slight rhythmic head movements, although palpebral reflex remained absent, and no reaction to visual, physical or auditory stimuli occurred (score 1); four goats showed slight tonic muscle spasms, primarily of the hind legs and tail (score 1), which disappeared by 20 minutes, after injection of diprenorphine. However, immobilisation was maintained throughout the trial until etorphine antagonism with diprenorphine, thereafter the animals all recovered uneventfully and showed no abnormal behaviour.

DISCUSSION

BIMU-8 led to an improvement in respiratory variables in etorphine-immobilised goats. Respiratory rate, SpO_2, PaO_2 and $P(A\text{-}a)O_2$ in the BIMU-8 group improved compared to the control and returned to pre-etorphine levels about 3–5 minutes after administration of BIMU-8. BIMU-8 also led to a transient decrease in blood pressure and an increased heart rate in the immobilised goats.

As expected, the intramuscular administration of etorphine induced rapid immobilisation in all goats. This immobilisation was accompanied by opioid-induced respiratory depression and compromise, as has been described previously in goats,[10,15,20] as well as other wild ungulates.[21–23]

BIMU-8 administration led to an improvement in f_R compared to the control. It was expected that intravenous administration of BIMU-8 would counter the etorphine-induced hypopnea due to its potential activation of $5\text{-}HT_{4a}$ receptors resulting in an increase in cAMP in the PBC.[7]

In addition, BIMU-8 caused an increase in $\dot{V}_E$. Thus, both an increase in f_R and $\dot{V}_E$, likely contributed to improving the blood gases in the immobilised goats. We also found that BIMU-8 administration led to an

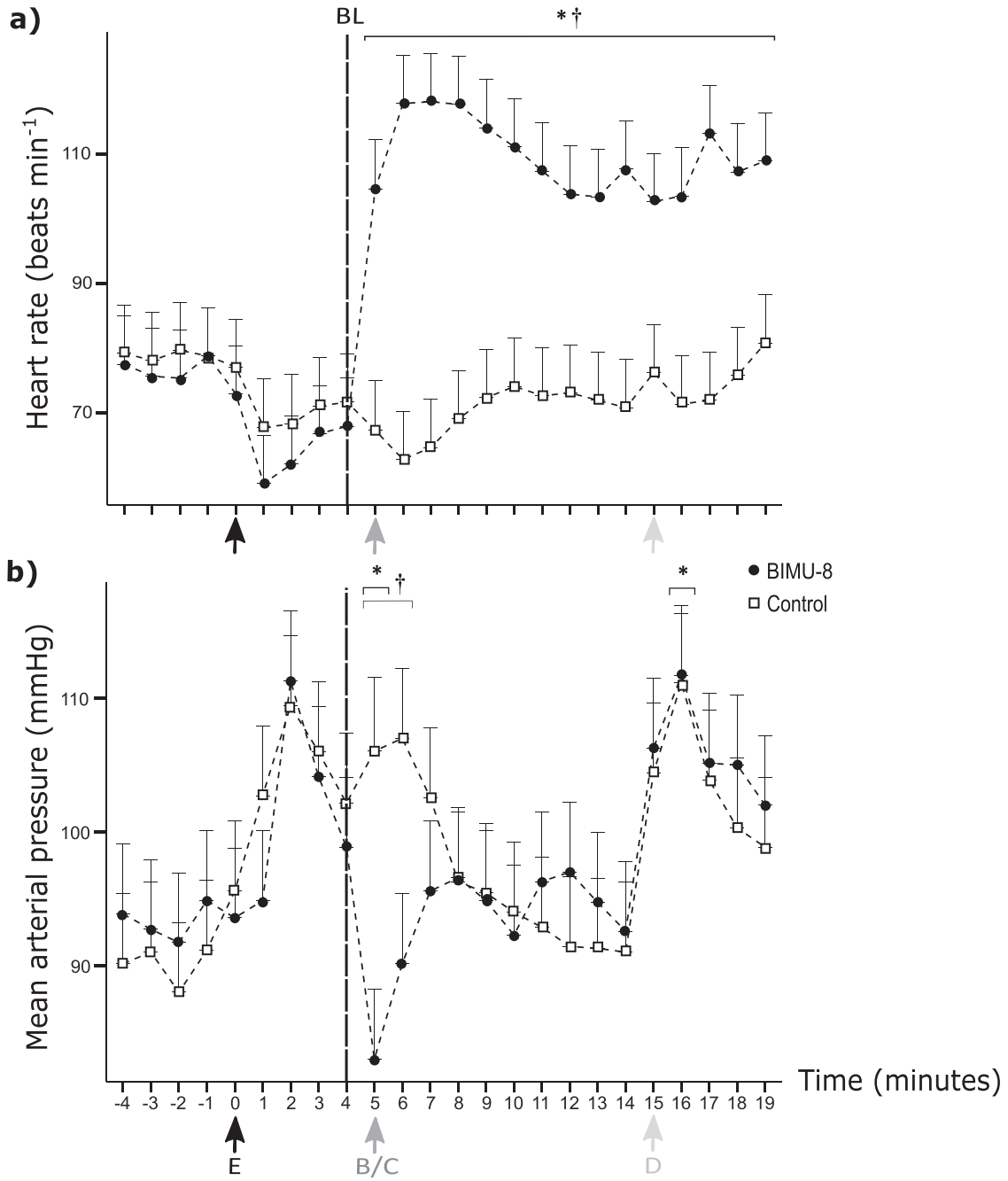

FIGURE 4 (**a-b**) Mean ± standard error of **(a)** heart rate (beats minute^{-1}) and **(b)** direct mean arterial blood pressure (mm Hg) in Boer goats ($n = 7$) administered 0.1 mg kg^{-1} etorphine hydrochloride IM and 3 mg kg^{-1} BIMU-8 or sterile water to the equal amount of mL BIMU-8 (control [C]) IV. †BIMU-8 treatment significantly different (all $p < 0.05$) from control at the respective time points. *BIMU-8 treatment (black dots) significantly different (all $p < 0.05$) from baseline (BL) at the respective time points. Arrows represent: E = etorphine injection (T0) (black arrow). BL = baseline (4 minutes after etorphine administration and 1 minute before injection of BIMU-8/control [T4] [dashed line]). B/C = administration of treatment (BIMU-8/control (T5) (dark gray arrow)). D = administration of diprenorphine, 15 minutes after etorphine administration (T15) (light grey arrow)

increase in PaO_2 and a decrease in $PaCO_2$ and the $P(A\text{-}a)O_2$ gradient. A decreased PaO_2 and an elevated $P(A\text{-}a)O_2$ gradient following etorphine administration has been attributed to a decrease in alveoli gas exchange caused by etorphine-induced pulmonary hypertension in goats.[10] Therefore, the administration of BIMU-8 may have also led to a decrease in pulmonary hypertension, with a subsequent improvement in gas exchange (decrease in $P(A\text{-}a)O_2$ gradient), which also would have contributed to the observed improvement in PaO_2. However, the mechanisms through which BIMU-8 may improve the $P(A\text{-}a)O_2$ gradient in etorphine-immobilised animals remains to be clarified. In humans, 5-HT4 receptors do not appear to be present in pulmonary arteries and are scarce in pulmonary veins.[24] However, the presence of 5-HT4 receptors in the pulmonary vein appears to be species-specific.[25] In the sheep isolated pulmonary vein, 5-HT4 receptors are abundant, and activation of these receptors mediates potent endothelium-independent relaxation.[25] It is therefore plausible that in our study, BIMU-8 led to a reduction in pulmonary pressure in the immobilised goats via direct activation of 5-HT4 receptors in the pulmonary vein.

Together with pulmonary vasodilation, we believe that BIMU-8 also led to systemic vasodilation, as MAP initially decreased after its administration. Systemic vasodilation induced by BIMU-8 may be beneficial in etorphine-immobilised animals as etorphine is known to cause an increase in systemic vascular resistance and hypertension.[26]

Within 2 minutes of administration, BIMU-8 led to a mean HR of 118 (±7) beats per minute, a 73% increase from baseline. Although this increase in HR might be part of a baroreflex response, given the initial fall, then return to pre-etorphine levels of MAP, there is evidence that suggests direct cardiac 5-HT4 receptor activation increases HR.[27,28] Activation of 5-HT4 receptors is thought to initiate a cascade via G-proteins, adenylyl cyclase, cAMP and then activating cyclic nucleotide-gated cation channels (HCN) in the sinus node.[28]

In human clinical trials using ECG, 5-HT4 agonists, such as prucalopride, revealed their pro-arrhythmic potential.[29] However, atria 5-HT4 receptors have thus far only been found in human and pig atria, while being absent from species such as the rat and rabbit.[30] We do not yet know whether goats express 5-HT4 receptors on cardiac myocytes. In rabbits, 5-HT4 receptors are not expressed in the heart, yet BIMU-8 caused tachycardia when administered to rabbits.[31]

Iqbal and Lemmens-Gruber[31] attributed this tachycardia in rabbits to BIMU-8′s interaction with cardiac ion channels, rather than receptors. BIMU-8 has pro-arrhythmic potential through blocking hERG, NaV1.5 and CaV1.2 cardiac ion channels, which leads to prolonged ECG QT-intervals and disrupted blood flow.[31] Thus, BIMU-8-related arrhythmias may occur either via direct activation of 5-HT4 receptors or cardiac ion channels.

In the present study, we did not detect any form of arrhythmias in the anaesthetised goats; the ECG readings appeared normal, without obvious evidence of arrhythmias such as premature ventricular contractions, torsades de pointes or heart blocks. However, our ECG trace was limited by poor resolution and finer assessment of the effects of BIMU-8 on the heart's electrical activity, and rhythmicity should be considered in future studies.

To better determine the effects of BIMU-8 on the cardiovascular system, further in-depth studies are required which should evaluate cardiac output, systemic and pulmonary vascular resistance and pressures. Additionally, the interaction between the cardio-respiratory systems, including variables that inform about oxygen delivery to, and extraction from, tissues, should be evaluated.

According to our immobilisation scores, BIMU-8 did not reverse immobilisation in the goats, although minor movements of the head and legs were observed in some animals. Manzke et al[7] found that activation of the 5-HT_{4a} receptors in rats did not alter fentanyl-induced analgesia. However, assessment of anaesthetic depth by scoring reflexes and movement, which is prone to subjective bias, may not be sufficiently sensitive to accurately assess the effects of BIMU-8 on anaesthetic depth in goats, and additional measures like electroencephalograms may be of value in the future.

Intravenous administration of BIMU-8 was performed for accurate assessment of the drug's effect. However, the ultimate goal is to prevent the negative respiratory effects of opioids from occurring when wildlife is immobilised. To achieve this goal, respiratory protective drugs need to be co-administered IM with opioids via a dart. It is possible that BIMU-8 may elicit slightly different, slower or less pronounced responses if it were administered IM.

Thus, it is important to determine whether intramuscular administration of BIMU-8, co-administered with etorphine, results in similar or different cardiorespiratory responses to those seen in this study.

CONCLUSION

In the etorphine-immobilised goats BIMU-8 caused significant changes in cardiovascular variables, but from the data collected it is not clear if these were beneficial or pathological, so further in-depth cardiovascular assessment is required. Without antagonising immobilisation, BIMU-8 attenuated etorphine-induced respiratory depression and improved gas exchange, which restored blood oxygenation and prevented hypercapnia from occurring. These effects indicate that BIMU-8 may be a good drug candidate for the treatment, or prevention, of etorphine-induced respiratory compromise in immobilised ungulates.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

Conceptualization of the study: Leith Meyer and Gabrielle Stalder. Performed experiments and data sampling: Nadine Tod and Leith Meyer. Statistical analysis: Hanna Rauch. Data interpretation: Nadine Tod, Hanna Rauch, Gabrielle Stalder, Stefan Boehmdorfer, Anna Haw, Hanno Gerritsmann, Johanna Painer and Leith Meyer. Funding acquisition,

resources: Leith Meyer and Stefan Boehmdorfer. Writing – original draft: Nadine Tod, Gabrielle Stalder, Anna Haw and Leith Meyer. Review and editing: Nadine Tod, Gabrielle Stalder, Hanna Rauch, Stefan Boehmdorfer, Anna Haw, Hanno Gerritsmann, Johanna Painer and Leith Meyer.

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