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

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Abstract

Neurophysiological studies have shown that serotonergic ligands that bind to 5-HT1A, 5-HT7 and 5-HT4 serotonin receptors in the brainstem have beneficial effects on respiratory neurons during opioid-induced respiratory depression. The effect of these ligands on respiratory function and pulmonary performance has not been studied. We therefore examined the effects of 8-OH-DPAT, an agonist of 5-HT1A and 5-HT7 receptors, and zacopride, an agonist of 5-HT4 receptors, to establish if these ligands would reverse opioid-induced respiratory depression and hypoxia without affecting the immobilizing properties of the opioid drug etorphine. When etorphine was used to sedate and immobilize goats it significantly decreased the goats respiratory rates ($P = 0.013$), percentage hemoglobin oxygen saturations ($P < 0.0001$) and PaO$_2$ ($F_{(10,70)} = 5.67$ $P < 0.05$) and increased PaCO$_2$ ($F_{(10,70)} = 3.87$, $P < 0.05$) and alveolar-arterial oxygen partial pressure gradient (A-a gradients)($F_{(10,70)} = 8.23$, $P < 0.0001$). Zacopride and 8-OH-DPAT, co-administered with etorphine, both attenuated the effects of etorphine; respiration rates did not decrease and the percentage hemoglobin oxygen saturations and PaO$_2$ remained elevated. Zacopride decreased the hypercapnia, indicating an improvement in ventilation, whereas 8-OH-DPAT did not affect the hypercapnia and therefore did not improve ventilation. The main beneficial effect of 8-OH-DPAT was on the pulmonary circulation; it improved oxygen diffusion, indicated by the normal A-a gradients, presumably by improving ventilation perfusion ratios. Neither zacopride nor 8-OH-DPAT reversed etorphine-induced catatonic immobilization. We conclude that serotonergic drugs that act on 5-HT1A, 5-HT7 and 5-HT4 receptors reverse opioid-induced respiratory depression and hypoxia without reversing catatonic immobilization.
Keywords: serotonin, etorphine, ventilation, A-a gradients.
**Introduction**

Opioids cause respiratory depression, a particular problem when they are used as analgesics (26; 27) and when they are used to immobilize wild herbivores (7; 16; 40). This respiratory depression may cause hypoxic damage to vital organs (31). Opioids affect the respiratory system mainly through their action on µ-opioid receptors on respiratory neurons in the pre-Bötzinger complex (14; 25), a collection of neurons in the brainstem that generate respiratory rhythm (39). The complex depends on neurotransmitters, including serotonin, for the modulation of respiratory rhythm (29). Serotonin enhances activity in respiratory neurons through its action on 5-HT₁A, 5-HT₄ and 5-HT₇ serotonin receptors (33). The contrasting actions of opioids and serotonin on respiratory neurons allows for the possibility that serotonergic ligands could alleviate the depressive action of opioids on these neurons.

That possibility has been realized in recent neurophysiological investigations. The serotonergic ligands 8-OH-DPAT, an agonist at 5-HT₁A and 5-HT₇ receptors, and buspirone, a partial agonist at the 5-HT₁A receptor, reversed morphine-induced depression of respiratory neurons in anaesthetized rats (37). BIMU8, an agonist at the 5-HT₄ receptor, reversed fentanyl-induced depression of respiratory neurons, importantly without reversing analgesia, in anaesthetized rats (25). However, although measurements of neuronal activity may reveal the potential of serotonergic ligands to influence respiration, determining whether such ligands actually improve respiratory function requires measurement of pulmonary performance in the whole animal. Also since serotonin receptors are widely distributed throughout the body (15), even if serotonergic
ligands improve pulmonary performance, they may generate adverse effects elsewhere in
the body which may negate that benefit. If they are to be used to alleviate opioid-induced
respiratory depression, they should not counteract the intentional effects of the opioids.

Mortality and morbidity resulting from the respiratory depression are major problems
when opioids are used to immobilize animals. We therefore set out to assess whether the
serotonergic ligands 8-OH-DPAT and zacopride could be employed to reverse such
depression, using the physiologically-relevant index of pulmonary function, namely
arterial blood gas status. Since opioids are used therapeutically much more often to
immobilize ungulates than to immobilize small animals, we used goats as an
experimental animal. As our opioid, we used the pharmacological agent preferred for
immobilization of ungulates, namely the morphine derivative etorphine, a potent agonist
of µ-opioid receptors. Concomitantly, we needed to establish if the serotonergic ligands
would influence etorphine-induced catatonia and sedation in the goats. We hypothesized
that 8-OH-DPAT, an agonist at 5-HT1A and 5-HT7 receptors, and zacopride, an agonist at
5-HT4 receptors and an antagonist at 5-HT3 receptors, would reverse opioid-induced
respiratory depression and hypoxia without reversing the opioid-induced catatonic
immobilization and sedation. Although our investigation was targeted to opioid-induced
immobilization, its outcomes clearly would have implications for respiratory depression
in patients under opioid analgesia.
Methods

Animals

Eight healthy adult female boer goats (*Capra hircus*), weighing 40±9 kg (mean ± SD), were used. They were housed in climatically-controlled indoor pens in Johannesburg, at an altitude of 1753 m, on a 12 hour light/dark cycle. They had water *ad libitum* and were fed on hay and sheep concentrate pellets. The procedures were approved by the University of the Witwatersrand’s Animal Ethics Screening Committee (clearance 2004/31/5).

Surgery

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

Etorphine hydrochloride (M99, Novartis, Johannesburg, South Africa) was injected i.m. at a dose of 0.06 mg.kg⁻¹. This dose adequately immobilized and sedated the goats for 30 min. Both 8-Hydroxy-2-dipropylaminotetralin hydrobromide (8-OH-DPAT, Tocris, Bristol, United Kingdom) and 4-Amino-N-1-azabicyclo[2.2.2]oct-3-yl-5-chloro-2-methoxybenzamide hydrochloride (Zacopride, Tocris, Bristol, United Kingdom) were used in their racemic form and were injected i.v. at a dose of 0.5 mg.kg⁻¹. This dose was established in a pilot dose-response study, as a mid-range dose which increased the respiratory rate in the goats under etorphine immobilization, without causing any harmful side effects. 8-OH-DPAT (5mg.ml⁻¹) and zacopride (10mg.ml⁻¹) both were dissolved in sterile injectable water (Kyron Laboratories, Johannesburg, South Africa).

**Experimental procedure**

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

Once the catheters were in place, the goat was moved into a trolley (0.6x1.5m) where it was restrained by a handler who held the horns. To measure arterial hemoglobin oxygen saturation and heart rate, a veterinary pulse oximeter (Nonin 9847V with 2000T animal transflectance sensor, Nonin Medical, North Plymouth, United States of America) was placed on the skin at the ventral tail base, and secured with adhesive tape. Saturation was measured to an accuracy of 3 % and heart rates to an accuracy of 2 beat.min⁻¹. A pressure transducer (1210 ICSensor, MSIsensor, Fairfield, United States of America) was connected to one arm of the three-way stop cock valve with 1.19 mm tubing (Portex Ltd, Kent, England) and the transducer attached to a processor constructed for us (School of Electrical Engineering, University of the Witwatersrand, Johannesburg, South Africa) to measure and log mean arterial pressure every 15 seconds to an accuracy of 2 mmHg. Rectal temperatures were measured with a thermocouple thermometer (BAT-12, Physitemp, Clifton, United States of America) to an accuracy of 0.2 ºC, and were used to calculate water vapour pressure in alveolar air. A digital stop-watch was used to record times to recumbency and respiratory rates. Recumbency was determined when a goat could no longer stand in a supine position on its own.
The etorphine injection induced immobilization and recumbency. The level of immobilization was assessed clinically by a veterinarian observing movement, neck tone and vocalization. The goats were held in sternal recumbency by a handler holding the horns so that the neck was aligned with the spinal column and the head was elevated above the thorax with the nose pointing downwards. This positioning allowed for unobstructed eructation of ruminal gas and open upper airways. After 30 min the action of etorphine was reversed by i.v. injection of 0.096mg.kg\(^{-1}\) diprenorphine hydrochloride (M5050, Novartis, Johannesburg, South Africa). Data recordings started 6 min before etorphine injection (injection time = 0 min) and continued for 40 min after injection. Heart rate, hemoglobin oxygen saturation, rectal temperature and respiration rate were recorded every two mins. Respiration rates were measured by counting breaths, visible by movement of the chest and abdominal wall, over a minute.

A 0.5ml carotid arterial blood sample was drawn 2 min before etorphine injection, at 6, 10, 20 and 30 min after etorphine injection, and 10 min after etorphine reversal. After each sample was drawn, the intra-arterial catheter was flushed with 5iu.ml\(^{-1}\) heparinized (Heparin, Intramed, Johannesburg, South Africa) saline. Directly after the sample was drawn, a blood gas analyzer (Roche OPTI CCA Analyzer + OPTI cassette B, Kat Medical, Johannesburg, South Africa) measured the partial pressure of oxygen (PaO\(_2\)) and carbon dioxide (PaCO\(_2\)) in the sample to an accuracy of 1.3 mmHg for PaO\(_2\) and 0.4 mmHg for PaCO\(_2\). At the end of each trial, the catheters were removed and a pressure bandage was placed over the carotid artery, for 6 hours, to prevent haematoma formation in the neck. Once the etorphine trials were completed, the goats were given i.v. injections
of $0.5\text{mg.kg}^{-1}$ 8-OH-DPAT and $0.5\text{mg.kg}^{-1}$ zacopride separately and without etorphine, to assess whether the serotonergic ligands alone had effects on the goats. At the end of the experiment all the goats were returned to stock.

All measurements were made indoors, between 8:00 and 13:00 at an ambient dry bulb temperature between 20°C and 22°C and relative humidity between 21% and 24%. Barometric pressures were measured to an accuracy of 0.1 mmHg, by the on-board barometer of the blood gas analyzer, which we had calibrated against a Fortin mercury barometer (On, F.D & Co Ltd, United Kingdom). Barometric pressure ranged from 628 mmHg to 634 mmHg.

**Data analysis**

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

For PaO$_2$, PaCO$_2$ and alveolar-arterial oxygen partial pressure gradients (A-a gradients), a two-way ANOVA followed by a Student Neuman Keuls (SNK) post-hoc test was used to test for differences between responses to pairs of injections and for differences between pre- and post-etorphine + water/ligand responses and pre-etorphine + water/ligand and post-diprenorphine administration in each trial. The A-a gradients were calculated for an open system (constant pressure) from the formula: FiO$_2$ (Pb – PH$_2$O) – PaCO$_2$ – PaO$_2$, where FiO$_2$ is the fractional inspired oxygen (0.209), Pb the measured barometric pressure (mmHg) and PH$_2$O the water vapour pressure of saturated air in the alveoli. PH$_2$O (mmHg) was calculated as $4.58 \exp \{ (17.27Tb)/(237.3 + Tb) \}$ (3), where Tb is the body temperature taken as per rectum. We assumed that the partial pressure of CO$_2$ in the alveoli was equal to the arterial partial pressure of CO$_2$ (13; 31; 35).
Results

Immobilization

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

Respiratory rate

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

**Percentage hemoglobin oxygen saturation**

Etorphine administration resulted in a significant (Student’s paired t-test, \(P < 0.0001\)) decrease in the saturation of arterial hemoglobin with oxygen over the 30 min of immobilization (Fig. 2). The decrease in saturation was greatest in the first 10 min of the immobilization. Saturation before etorphine administration was 96±3 % (n = 8) and dropped to as low as 75±7 % (n = 8) after 4 min, with a gradual increase thereafter over time. After diprenorphine injection the saturation returned to near pre-injection values (Student’s paired t-test, \(P = 0.5\)). Although saturations significantly decreased after the administration of etorphine + zacopride (Student’s paired t-test, \(P = 0.0025\)) and etorphine + 8-OH-DPAT (Student’s paired t-test, \(P = 0.0002\)), both zacopride and 8-OH-DPAT attenuated the etorphine-induced decrease in saturation. Over the entire immobilization period, saturation in the goats that received etorphine + zacopride was significantly (F = 7.18, \(P < 0.05\)) higher than that evident when they received etorphine +
water. Saturation in the goats that received etorphine + 8-OH-DPAT was significantly (F = 10.76, P = 0.0015) higher than that when they received etorphine + water only over the first 10 min interval after administration. Zacopride (Student’s paired t-test, P = 0.75) did not alter the return of saturations to pre-injection levels after diprenorphine administration, whereas saturation of the goats that received 8-OH-DPAT + etorphine remained moderately depressed (Student’s paired t-test, P = 0.02).

Partial pressure of oxygen

Figure 3 shows the effect of administration of etorphine, with and without the serotonergic ligands, on arterial pressure of oxygen (PaO₂). PaO₂ was 69±4 mmHg (n = 8) before etorphine administration. Following the injection of etorphine + water, PaO₂ dropped to below 50 mmHg after 6 min. The drop following etorphine + water was significant (F(10,70) = 5.67 P < 0.05) over the first 20 min of immobilization. Thereafter the PaO₂ gradually increased, and returned to pre-injection values (F(10,70) = 5.66 P = 0.5) after diprenorphine injection. Zacopride and 8-OH-DPAT attenuated, but did not fully abolish, the etorphine-induced decrease in PaO₂ and even though the PaO₂ values decreased when zacopride and 8-OH-DPAT were injected with etorphine, both drugs maintained significantly (F(10,70) = 5.67, P < 0.05) higher levels of PaO₂ in the goats in the first 10 min of immobilization. Neither zacopride (F(10,70) = 5.67, P = 0.64) nor 8-OH-DPAT (F(10,70) = 5.67, P = 0.95) affected the return of PaO₂ values to pre-injection values after diprenorphine administration.
Partial pressure of carbon dioxide

Administration of etorphine also resulted in a significant \( F(10,70) = 3.87, P < 0.05 \) increase in the PaCO₂ throughout the immobilization period (Fig.4). PaCO₂ was 31±2 mmHg (n = 8) before etorphine administration. The highest PaCO₂ (41±5 mmHg) occurred 6 min after the etorphine + water injection and gradually decreased over time and returned to pre-injection values after diprenorphine injection \( F(10,70) = 3.87, P = 0.94 \). The co-administration of 8-OH-DPAT with etorphine had no beneficial effect, and the PaCO₂ levels remained significantly \( F(10,70) = 3.87, P < 0.001 \) elevated throughout the immobilization. Zacopride co-administration significantly attenuated the rise in PaCO₂ caused by etorphine. The PaCO₂ for etorphine + zacopride was significantly \( F(10,70) = 3.87, P < 0.05 \) lower than that for etorphine + water and etorphine + 8-OH-DPAT in the first 20 min of the immobilization period. Zacopride \( F(10,70) = 3.87, P = 0.93 \) did not alter the return of PaCO₂ values to pre-injection values after diprenorphine administration, whereas in the etorphine + 8-OH-DPAT trial, PaCO₂ values did not return to pre-injection values and remained moderately elevated \( F(10,70) = 3.87, P < 0.05 \).

Alveolar-arterial oxygen partial pressure gradient

Figure 5 shows the effect of etorphine administration, with and without co-administration of the serotonergic ligands, on a derived variable, namely alveolar – arterial gradient in the partial pressures of oxygen. The gradient was 21±3 mmHg (n = 8) before administration of etorphine. When etorphine + water was injected there was a significant \( F(10,70) = 8.23, P < 0.0001 \) increase in the A-a gradient, which resolved progressively
during the time course of the immobilization. Co-administration of 8-OH-DPAT with etorphine abolished the increase in the gradient ($F_{(10,70)} = 8.23, P = 0.5$), and indeed the gradient remained below the pre-injection gradient throughout immobilization. Co-administration of zacopride attenuated ($F_{(10,70)} = 8.23, P = 0.003$) but did not abolish the effects of etorphine on the A-a gradient ($F_{(10,70)} = 8.23, P < 0.002$). After administration of diprenorphine the A-a gradients dropped significantly below pre-injection values in the etorphine + water ($F_{(10,70)} = 8.23, P = 0.0004$) and the 8-OH-DPAT + etorphine ($F_{(10,70)} = 8.23, P = 0.005$) trials.

**Heart rate**

Figure 6 shows the effects of administrating etorphine, with and without serotonergic ligands, on heart rate. Heart rate was 67±5 beats.min$^{-1}$ (n = 8) before etorphine administration. Over the time course of the immobilization, heart rate decreased following etorphine administration, whether or not the ligands were co-administered. In contrast to its effect on respiratory variables, etorphine administration did not affect heart rate immediately. Heart rate was unchanged for at least the first 8 min after etorphine administration. Thereafter, the decline in heart rate was attenuated by co-administration of zacopride, but accentuated by co-administration of 8-OH-DPAT. After the second 10 min interval, heart rate was significantly ($F_{(2,7)} = 0.33, P < 0.001$) decreased following 8-OH-DPAT co-administration, and increased ($F_{(2,7)} = 0.33, P < 0.01$) following zacopride co-administration, compared to the heart rates which followed etorphine co-administration with water. In the etorphine + water and etorphine + zacopride trials the heart rates returned to pre-injection rates after diprenorphine administration, whereas the
heart rates in the etorphine + 8-OH-DPAT trial remained significantly (Student’s paired t-test, \( P < 0.0001 \)) lower than the pre-injection rates.

*Mean arterial pressure*

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

At a dose at which it immobilized goats, the opioid etorphine caused marked respiratory depression. Symptomatically, this depression was evident as a decrease in respiratory rate to about half of the rate before etorphine administration. The respiratory rate remained low throughout the immobilization period, but, alone, it did not reveal the respiratory status of the animals. Directly after the administration of etorphine, and up until 10 min after injection, respiratory depression was the most severe; the animals became clinically hypoxic, taken as PaO₂ < 60mmHg and percentage arterial hemoglobin saturation < 85% (8). Hypoxia resulted both from a decrease in the ventilation, indicated by an increase in PaCO₂, and a decrease in diffusion, presumably via a ventilation-perfusion mismatch, indicated by an increase in the alveolar-arterial gradient in oxygen partial pressure. After 10 min, there was a gradual increase in both the PaO₂ and the percentage oxygen hemoglobin saturation, which was brought about predominantly by an improvement in diffusion (compare Figs 4 and 5).

That opioids depress respiration is well known. What we have shown, we believe for the first time, is that the depressed respiratory function can be reversed substantially by administration of serotonergic ligands. Co-administration of zacopride or 8-OH-DPAT with etorphine improved the respiratory function of the goats, such that PaO₂ and arterial hemoglobin saturation remained above levels defining clinical hypoxia. The ligands, which act at different 5-HT receptors, reversed respiratory depression by different physiological mechanisms. Zacopride attenuated the decrease in the respiratory rate and decreased the hypercapnia, indicating improved ventilation. 8-OH-DPAT also attenuated
the decrease in the respiratory rate but did not improve ventilation, because PaCO₂ remained elevated (Fig. 4). The main beneficial effect of 8-OH-DPAT was on the pulmonary circulation; it improved diffusion, as indicated by the restoration of normal differences between alveolar and arterial partial pressures of oxygen (Fig. 5), presumably by improving ventilation-perfusion ratios. Zacopride also partially restored the alveolar-arterial gradient but its effect was not as great as that of 8-OH-DPAT. In addition to the deleterious effects on the respiratory system, the opioid also affected the cardiovascular status of the goats, by inducing bradycardia and transient hypertension (Figs 6 and 7). The serotonergic ligands influenced those cardiovascular effects too. Zacopride abolished the hypertension whereas 8-OH-DPAT transiently exacerbated the etorphine-induced biphasic changes in mean arterial pressure (Fig. 7). Similarly, zacopride reduced, and 8-OH-DPAT enhanced, the bradycardia (Fig. 6).

Both serotonergic ligands improved respiratory function and affected the cardiovascular status without reversing catatonic immobilization, a necessity given that the primary use of etorphine is chemical immobilization of animals. Indeed the co-administration of both zacopride and 8-OH-DPAT with etorphine significantly decreased the time it took for the goats to become recumbent. Thus we have shown that the serotonergic ligands zacopride and 8-OH-DPAT, acting through physiologically distinct mechanisms, improved the respiratory status of goats immobilized with the opioid etorphine, without reversing catatonic immobilization, and zacopride also improved the cardiovascular status of the goats.
It should be noted that the laboratory in which we conducted our experiments was situated at an altitude at which the respiratory status of even intact animals is somewhat different to that at sea level; arterial partial pressure of oxygen, for example, was 70 ± 4 mmHg in the goats before immobilization. However, we have no reason to suspect that the effects of the agents on the respiratory system would differ at altitudes lower than ours, though actual values of variables like the partial pressure of blood gases and the oxygen hemoglobin saturation would differ. Another potential limitation of our study is that zacopride and 8-OH-DPAT are ligands that act on more than one serotonin receptor. Where we have drawn conclusions about the effects of zacopride or 8-OH-DPAT on one specific receptor, we have based these conclusions on the results from previous studies which have investigated the function of specific 5-HT receptors ligands.

Serotonergic receptors in neuronal pathways play important roles in the modulation of respiratory rhythm (33). Many studies have examined the effects of serotonin and its congeners on the function of respiratory neurons, specifically during sedative-induced compromise of those neurons. Indeed, the actions of the ligands which we employed have been explored in that context. Sahibzada et al. (37) showed that 8-OH-DPAT reversed the morphine-induced suppression of neuronal activity in anaesthetized rats and Lalley et al. (22) used 8-OH-DPAT to reverse pentobarbital- and ketamine-induced suppression of respiratory neurons in cats. Richter et al. (33) claimed that the effect of 8-OH-DPAT on the neurons generating respiratory rhythm results from its agonism of 5-HT7 receptors. They proposed that the reversal of morphine-induced neuronal suppression observed by Sahibzada et al. (37) depended on 8-OH-DPAT’s action on 5-HT7 receptors and not, as
Sahibzada et al. (2000) had believed, on 5-HT1A receptors. Even if the action of 8-OH-DPAT is mediated by the 5-HT7 receptors, 5-HT1A receptors also are facilitatory in reversing morphine-induced suppression of respiratory neurons, because buspirone, a 5-HT1A agonist that has no effect on the 5-HT7 receptor (33), also reversed the suppression (37). 8-OH-DPAT may well improve the activity of the neurons generating respiratory rhythm through its action on both the 5-HT1A and 5-HT7 receptors. We believe that 8-OH-DPAT increased respiratory frequency, in our goats, through its action on respiratory neurons, rather than through the enhancement of the hypoxic drive that the goats experienced after etorphine administration. This belief is supported by the finding that 8-OH-DPAT did not increase respiratory frequency or ventilation rate in hypoxic goats (20).

8-OH-DPAT’s activation of 5-HT7 receptors provokes c-AMP formation (33) in respiratory neurons, which then stimulates the respiratory rhythm (2). It is not clear how 8-OH-DPAT’s concomitant activation of the 5-HT1A receptors could improve respiratory rhythm, though Lalley and colleagues (22) found that, in anaesthetized cats, 8-OH-DPAT’s action on 5-HT1A receptors prevented prolonged discharge of early inspiratory neurons. In another study, Lalley et al. (21) showed that the effect of 8-OH-DPAT on inspiratory neurons is dose-dependent. At lower doses, (10-50 µg.kg⁻¹), 8-OH-DPAT increased the frequency of phrenic nerve discharges in anaesthetized cats, but higher doses (50 and 90µg.kg⁻¹) suppressed phrenic nerve discharges. In a similar and more recent study (34), phrenic nerve discharges were decreased even when 20 µg.kg⁻¹ of 8-OH-DPAT was injected intravenously in cats. We used a much higher dose (500 µg.kg⁻¹)
of 8-OH-DPAT in our goats, and we did not observe any effects consistent with depression of respiratory neurons. Sahibzada et al. (37) also found that 8-OH-DPAT had no depressant effects on rat respiratory neurons when injected at a dose of 100 μg.kg⁻¹.

In contrast to the uncertainties about the action of 8-OH-DPAT on respiratory neurons, the action of zacopride on such neurons seems to derive unambiguously from its agonism of 5-HT4 receptors, rather than antagonism of 5-HT3 receptors. Zacopride has been shown to be an agonist of the 5-HT₄(a) receptor isoform (28), and Manzke et al. (25) discovered that inspiratory neurons in the pre-Bötzinger complex host both 5-HT₄(a) and µ-opioid receptors. Stimulation of the µ-opioid receptors would decrease cAMP in inspiratory neurons (2) and consequently decrease inspiratory drive, whereas stimulation of the 5-HT₄(a) receptors would increase cAMP and thus increase inspiratory drive (25).

In contrast to the degree of investigation on the actions of serotonergic ligands on respiratory neurons, as far as we can establish no-one has investigated the actions of serotonergic ligands on the function of the effector organs in the respiratory system. It is far from obvious how activity on neurons responsible for respiratory rhythms would translate into effects on the clinically-important phenomena of hypoxia and hypercapnia induced by opioids, nor, as we think we have discovered, is it guaranteed that improvement of oxygenation results from actions on respiratory neurons. We have demonstrated that, in goats subjected to opioid immobilization, although 8-OH-DPAT improved respiratory rate, it did not improve alveolar ventilation; hypercapnia did not decrease when 8-OH-DPAT was co-administered with etorphine. Nevertheless, 8-OH-
DPAT co-administered did improve PaO2. We believe that this increase in the PaO2 depended on 8-OH-DPAT countering the effects of the opioid on the pulmonary vasculature. Opioids decrease PaO2 both by reducing alveolar ventilation and by disrupting pulmonary blood perfusion. Pulmonary perfusion decreases under the influence of opioids both because hypoxia causes pulmonary vasoconstriction (31), and because opioids directly cause pulmonary vasoconstriction (19; 38). They do so by inducing the release of histamine in the lungs (17; 26) and by activating the sympathetic nervous system centrally (36). We believe that 8-OH-DPAT improved blood oxygenation primarily by reducing pulmonary blood shunting, through its serotonergic effects on the pulmonary vasculature.

Serotonin has a strong vasoactive effect on the pulmonary vasculature (15). In goats serotonin causes vasoconstriction in the pulmonary arteries and vasodilation in the pulmonary veins (10). Serotonin-induced pulmonary vasoconstriction appears to be brought about mainly by the activation of 5-HT2A receptors (24), to which our ligands did not bind, and pulmonary venodilation by the activation of 5-HT4 receptors (11). Although no-one appears to have explored the effects of 5-HT7 receptor activation in the goat’s pulmonary vasculature, we believe that 8-OH-DPAT may have improved the pulmonary perfusion that had been compromised by opioid administration, through its action on 5-HT7 receptors. Our belief is supported by the identification of 5-HT7 receptors in the pulmonary vasculature of many other mammalian species (4; 42), and the observation that 5-HT7 receptor activation causes smooth muscle relaxation (42; 43). There also is evidence that 5-HT7 receptors may be involved in pulmonary vasodilation in rabbits (30).
Zacopride causes venodilation in the pulmonary vasculature through its action on 5-HT₄ receptors (11). Venodilation would increase pulmonary perfusion, and although any increase in pulmonary perfusion would have contributed to improving oxygenation, in our goats zacopride acted primarily to improve ventilation, in so doing reducing hypercapnia and improving both PaO₂ and hemoglobin oxygen saturation. It seems likely that the activity of zacopride on pre-Bötzinger neurons, compromised by opioid administration, accounted for the restoration of ventilation.

Although there have been several studies showing that serotonergics ligands act on respiratory networks in the central nervous system (21-23; 25; 37), we believe that our study is one of the few showing the effects of serotonergics on blood gases, and that it is the first study showing that serotonergics reverse opioid-induced respiratory depression and hypoxia, without reversing catatonic immobilization, an outcome that mirrors, for the whole animal, the conclusion of Manzke et al (2003) that a serotonergic ligand can excite respiratory neurons without affecting those involved in analgesia. We also have shown that the effect of serotonergics on the pulmonary vasculature plays an important role in influencing respiratory status, in addition to effects mediated by central respiratory networks. In addition to their effects on the pulmonary vasculature, the ligands also affect the general circulation, with zacopride improving the deleterious consequences of the opioid on blood pressure and heart rate, and 8-OH-DPAT worsening them, but only mildly and transiently.
Opioids are used in veterinary practice and game management to immobilize mammals (16; 40). They induce a catatonic immobilization by acting on localized areas in the central nervous system (41). In the rat, at least, the most prominent of these areas are the nucleus raphe pontis (1; 5; 6; 41; 44) and the nucleus accumbens (12). Both these nuclei contain serotonergic receptors (44), and serotonin enhances opioid-induced catatonia (6; 12; 44). To the best of our knowledge, no-one has identified which serotonin receptors are involved in such enhancement. We have shown that both zacopride and 8-OH-DPAT enhanced opioid-induced catatonia, in that both reduced time to recumbency in our goats, when co-administered with etorphine. Subsequently, though zacopride somewhat reduced, rather than enhanced, the sedative effects of etorphine. This finding may be explained if zacopride, through its 5-HT₃ antagonistic effects, reversed the effects of kappa opioid receptors (18), thereby resulting in a decrease in opioid-induced hypotonic immobility (9). It would seem that more than one serotonergic receptor mediates the enhancement of opioid-induced immobilization, but because these ligands each act on two 5-HT receptor types, we are unable to draw any conclusions as to which receptors are involved. We do know that neither ligand, at least at the dose we used, brought about immobilization in its own right.

We postulate that the key serotonergic receptors involved in combating opioid-induced respiratory depression, at least in goats, are the 5-HT₄ and 5-HT₇ receptors, but positive identification of the receptors will require further studies with specific ligands. However, until we also know what serotonergic receptor is responsible for improving opioid-induced catatonic immobilization, we should not conclude that a specific receptor ligand
would be the most putative therapeutic agent to improve both immobilization and respiratory welfare.

In summary we have shown that the serotonergic ligands improve blood oxygenation, in goats with respiration depressed by opioid administration, both by improving ventilation and by improving oxygen diffusion, we believe, by improving pulmonary perfusion. Further studies are required to identify the mechanisms involved, and will require measurements of pressures and flows in the respiratory system. Also, although our focus has been reduction of morbidity and mortality resulting from respiratory depression in animals immobilized by opioid administration, and although extrapolation between species should be made with caution, we feel that we also have provided more evidence that serotonergic ligands might be useful in reversing respiratory depression in patients under opioid analgesia or anesthesia, without interfering with the intentional effects of the opioids.
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Respiratory rate (breaths.min^{-1})(mean ± SD, n = 8) of goats injected (solid arrow, time = 0 min) I.M. + I.V. with etorphine + water (□), etorphine + zacopride (●) and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30min,) indicates I.V. injection of diprenorphine. a, $P < 0.05$ etorphine + zacopride vs. etorphine + water and b, $P < 0.05$ etorphine + 8-OH-DPAT vs. etorphine + water, one-way ANOVA with post-hoc SNK test on areas between the curves. c, $P < 0.025$ etorphine + water pre-injection vs post-injection, Student’s paired t-test. Respiratory rates were not significantly different between the trials before the agents were injected ($F = 3.1, P = 0.19$).
Figure 2. Drug effects on percentage hemoglobin saturation of arterial blood by oxygen

Percentage saturation of arterial hemoglobin by oxygen (mean ± SD, n = 8) of goats injected (solid arrow, time = 0 min) I.M. + I.V. with etorphine + water (□), etorphine + zacopride (●) and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30min) indicates I.V. injection of diprenorphine. a, \( P < 0.0125 \) etorphine + zacopride vs. etorphine + water and b, \( P < 0.0125 \) etorphine + 8-OH-DPAT vs. etorphine + water, one-way ANOVA with post-hoc SNK test on areas between the curves. c, \( P < 0.025 \) etorphine + water pre-injection vs post-injection and d, \( P < 0.025 \) etorphine + 8-OH-DPAT pre-injection vs post-reversal, Student’s paired t-test. Saturations were not significantly different between the trials before the agents were injected (\( F = 0.1., P = 0.9 \)).
Figure 3. Drug effects on arterial partial pressure of oxygen

Arterial partial pressure of oxygen (PaO₂ mmHg) (mean ± SD, n = 8) of goats injected (solid arrow, time = 0 min) I.M. + I.V. with etorphine + water (□), etorphine + zacopride (●) and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30min) indicates I.V. injection of diprenorphine. a, $P < 0.05$ etorphine + zacopride vs. etorphine + water and b, $P < 0.05$ etorphine + 8-OH-DPAT vs. etorphine + water. c, $P < 0.05$ etorphine + water pre-injection vs post-injection; d, $P < 0.05$ etorphine + zacopride pre-injection vs post-injection and e, $P < 0.05$ etorphine + 8-OH-DPAT pre-injection vs post-injection, two-way ANOVA with post-hoc SNK test. PaO₂ values were not significantly different between the trials before the agents were injected ($F_{10,70} = 5.67, P > 0.05$).
Figure 4. Drug effects on arterial partial pressure of carbon dioxide

Arterial partial pressure of carbon dioxide (PaCO₂ mmHg) (mean ± SD, n = 8) of goats injected (solid arrow, time = 0 min) I.M. + I.V. with etorphine + water (□), etorphine + zacopride (●) and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30min) indicates I.V. injection of diprenorphine. a, \(P < 0.05\) etorphine + zacopride vs. etorphine + water, b, \(P < 0.05\) etorphine + 8-OH-DPAT vs. etorphine + water and c, \(P < 0.05\) etorphine + zacopride vs. etorphine + 8-OH-DPAT. d, \(P < 0.05\) etorphine + water pre-injection vs post-injection; e, \(P < 0.05\) etorphine + zacopride pre-injection vs post-injection and f, \(P < 0.05\) etorphine + 8-OH-DPAT pre-injection vs post-injection/reversal, two-way ANOVA with post-hoc SNK test. PaCO₂ values were not significantly different between the trials before the agents were injected (\(F_{(10,70)} = 3.87, P > 0.05\)).
Figure 5. Drug effects on the alveolar arterial oxygen partial pressure gradient

Alveolar arterial oxygen partial pressure gradient (A-a gradient, mmHg) (mean ± SD, n = 8) of goats injected (solid arrow, time = 0 min) I.M. + I.V. with etorphine + water (□), etorphine + zacopride (●) and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30 min) indicates I.V. injection of diprenorphine. a, $P < 0.05$ etorphine + zacopride vs. etorphine + water, b, $P < 0.05$ etorphine + 8-OH-DPAT vs. etorphine + water and c, $P < 0.05$ etorphine + zacopride vs. etorphine + 8-OH-DPAT. d, $P < 0.05$ etorphine + water pre-injection vs post-injection/reversal; e, $P < 0.05$ etorphine + zacopride pre-injection vs post-injection and f, etorphine + 8-OH-DPAT pre-injection vs post-reversal, two-way ANOVA with post-hoc SNK test. Values were not significantly different between the trials before the agents were injected ($F_{(10,70)} = 8.23, P > 0.05$).
Figure 6. Drug effects on heart rates

Heart rates (beats.min\(^{-1}\)) (mean ± SD, n = 8) of goats injected (solid arrow, time = 0 min) I.M. + I.V. with etorphine + water (□), etorphine + zacopride (●) and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30min) indicates I.V. injection of diprenorphine. a, \(P < 0.0125\) etorphine + zacopride vs. etorphine + water and b, \(P < 0.0125\) etorphine + 8-OH-DPAT vs. etorphine + water, one-way ANOVA with post-hoc SNK test on areas between the curves. c, \(P < 0.025\) etorphine + 8-OH-DPAT pre-injection vs post-reversal, Student’s paired t-test. Heart rates were not significantly different between the trials before the agents were injected (\(F = 0.03, P = 0.7\)).
Figure 7. Drug effects on mean arterial pressure

Mean arterial pressures (mmHg) (mean ± SD, n = 8) of goats injected (solid arrow, time = 0 min) I.M. + I.V. with etorphine + water (□), etorphine + zacopride (●) and etorphine + 8-OH-DPAT (▲). Dashed arrow (time = 30min) indicates I.V. injection of diprenorphine. a, $P < 0.0125$ etorphine + zacopride vs. etorphine + water, b, $P < 0.0125$ etorphine + 8-OH-DPAT vs. etorphine + water and c, $P < 0.0125$ zacopride + etorphine vs. 8-OH-DPAT + etorphine, one-way ANOVA with post-hoc SNK test on areas between the curves. d, $P < 0.025$ etorphine + water pre-injection vs post-reversal; e, $P < 0.025$ etorphine + zacopride pre-injection vs post-reversal and f, $P < 0.025$ etorphine + 8-OH-DPAT pre-injection vs post-reversal, Student’s paired t-test. Mean arterial pressures were not significantly different between the trials before the agents were injected ($F = 0.41, P = 0.67$).