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

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Meyer, Leith C. R., Andrea Fuller, and Duncan Mitchell. Zacopride and 8-OH-DPAT reverse opioid-induced respiratory depression and hypoxia but not catatonic immobilization in goats. Am J Physiol Regul Integr Comp Physiol 290: R405–R413, 2006.—Neurophysiological studies have shown that serotonergic ligands that bind to 5-HT1A, 5-HT7, and 5-HT4 serotonin receptors in brain stem 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-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), an agonist of 5-HT1A and 5-HT7 receptors, and zacopride, an agonist of 5-HT4 receptors, to establish whether these ligands would reverse opioid-induced respiratory depression and hypoxia without affecting the immobolizing properties of the opioid drug etorphine. When etorphine was used to sedate and immobilize goats, it significantly decreased respiratory rate (P = 0.013), percent hemoglobin oxygen saturation (P < 0.0001), and arterial oxygen partial pressure [Pao2; F(10,70) = 5.67, P < 0.05] and increased arterial carbon dioxide partial pressure [Paco2; 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, coadministered with etorphine, both attenuated the effects of etorphine; respiration rates did not decrease, and percent hemoglobin oxygen saturation and PaO2 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 depressive and hypoxia without reversing catatonic immobilization.

serotonin; etorphine; ventilation; alveolar-arterial oxygen partial pressure gradients

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 brain stem that generate respiratory rhythm (39). The complex depends on neurotransmitters, including serotonin (5-HT), 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 allow for the possibility that serotonergic ligands could alleviate the depressive action of opioids on these neurons.

This possibility has been realized in recent neurophysiological investigations. The serotonergic ligands 8-hydroxy-2-(di-n-propylamino)tetralin (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 anesthetized rats (37). BIMU8, an agonist at the 5-HT1B receptor, reversed fentanyl-induced depression of respiratory neurons, importantly, without reversing analgesia, in anesthetized 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, because 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 that 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 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. Because 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 whether 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.

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methods

animals. eight healthy adult female boer goats (capra hircus),
weighing 40 kg (mean, sd 9), were used. they were housed in
climatically controlled indoor pens in johannesburg, at an altitude of
1,753 meters, on a 12:12-h light-dark cycle. they had access to 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 intramuscular injection of 2.5 mg/kg ketamine (anaket; bayer
animal health, johannesburg, south africa) and 0.04 mg/kg medeto-
midine (domitor; novartis, johannesburg, south africa). the goats
then were intubated, and anesthesia was maintained with 1–3%
halothane (fluothane; astra zeneca pharmaceuticals, johannesburg,
south africa) in oxygen. when inhalation anesthesia was stable, 0.2
mg/kg atipamezole hydrochloride (antisedan; novartis) was injected
intramuscularly 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 and roby (32), to allow for subsequent repetitive
arterial catheterization in conscious animals. after surgery, a pressure
bandage was placed over the site for 24 h. the animals were given a
month to recover before the experimental trials commenced.

Drugs. etorphine hydrochloride (m99; novartis) was injected in-
tramuscularly at a dose of 0.06 mg/kg. this dose adequately immo-
obilized and sedated the goats for 30 min. both 8-Oh-dPat hydro-
bromide (tocris, bristol, uk) and 4-amino-N-1-azabicyclo[2.2.2]
oct-3-yl-5-chloro-2-methoxybenzamide hydrochloride (zacopride; tocris)
were used in their racemic form and were injected intrave-
nously at a dose of 0.5 mg/kg. this dose was established in a pilot
dose-response study as a midrange dose that increased the respiratory
rate in the goats under etorphine immobilization without causing any
harmful side effects. both 8-Oh-dPat (5 mg/ml) and zacopride (10
mg/ml) were dissolved in sterile injectable water (kyron laboratories,
Johannesburg, south africa).

Experimental Procedures. the experiment consisted of three trials
in which each goat received etorphine + water (control), etorphine +
zacopride, and etorphine + 8-Oh-dPat, in random order, at weekly
intervals. the goats were weighed 2 days before each trial and were
starved for 24 h 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 0.9%
NaCl; adcock Ingram, johannesburg, south africa) for subsequent
drug injection. local anesthetic (2 ml of lignocaine; bayer animal
health) 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 4 mm long, into the carotid artery. a
three-way stopcock valve (Sabex, 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
(0.6 × 1.5 m), 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, mn) 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 beats/min. a pressure transducer (1210 ICSensor;
Msi sensors, fairfield, nj) was connected to one arm of the three-
way stopcock valve with 1.19-mm tubing (Portex, kent, uk), and the
transducer was attached to a processor constructed for us (School of
electrical engineering, university of the witwatersrand) to measure
and log mean arterial pressure every 15 s to an accuracy of 2 mmHg.
rectal temperatures were measured with a thermocouple thermometer
(BAT-12; physitemp instruments, clifton, nj) to an accuracy of
0.2°C and were used to calculate water vapor pressure in alveolar air.
a digital stopwatch 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 downward. this positioning
allowed for unobstructed eructation of ruminal gas and open upper
airways. after 30 min, the action of etorphine was reversed by
intravenous injection of 0.096 mg/kg diprenorphine hydrochloride
(M5050; novartis). 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 tempera-
ture, and respiration rate were recorded every 2 min. respiration rates
were measured by counting breaths, visible by movement of the chest
and abdominal wall, over a minute.

a 0.5-ml carotid arterial blood sample was drawn 2 min after
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 5 IU/ml heparinized (heparin;
intramed, johannesburg, south africa) saline. directly after the sam-
ple was drawn, a blood gas analyzer (roche opti CCA analyzer +
OPTI cassette B; kat medical, johannesburg, south africa) was used
to measure the arterial partial pressure of oxygen (PaO2), carbon
dioxide (PaCO2), and hemoglobin concentration (Hb). PaO2 and
PaCO2 were measured at the end of each trial, the catheters were
removed, and a pressure bandage was placed over the carotid artery
for 6 h to prevent hematoma formation in the neck. once the etorphine
trials were completed, the goats were given intravenous injections of
0.5 mg/kg 8-Oh-dPat and 0.5 mg/kg zacopride separately and
without etorphine, to assess whether the serotonergic ligands alone
did not affect the goats. at the end of the experiment, all of the goats
were returned to stock.

All measurements were made indoors, between 0800 and 1300,
at an ambient dry bulb temperature between 20 and 22°C and relative
humidity between 21 and 24%. barometric pressures were measured
to an accuracy of 0.1 mmHg by using the on-board barometer of the
blood gas analyzer, which we had calibrated against a fornt mercury
barometer (Russel scientific Instruments, dereham, UK). barometric
pressure ranged from 628 to 634 mmHg.

Data analysis. we used GraphPad prism version 4.00 for Windows
(GraphPad Software, san diego, CA) and statistica 99 edition (Stat-
Soft, tula, ok) for statistical analyses. all results were reported as
means, SD, and 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 (preetorphine +
water/ligand administration), for the first, second, and third 10-min
intervals and the entire 30 min after etorphine + water/ligand
administration, and for the 10 min after diprenorphine administration.
A one-way ANOVA followed by a Student-Newman-Keuls (SNK) post
hoc test was used to test for differences in 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 postetorphine + water/ligand administration, and between
preetorphine + water/ligand and postdiprenorphine administration.
Bonferroni corrections were applied where necessary.

For PaO2, PaCO2, and alveolar-arterial oxygen partial pressure gra-
dients (A–a gradients), a two-way ANOVA followed by a SNK post
hoc test was used to test for differences between responses to pairs of

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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-h gradients were calculated for an open system (constant pressure) from the formula 
\[
F_iO_2(P_b - P_{H_2O}) - P_{CO_2} - P_{H_2O},
\]
where \(F_iO_2\) is the fractional inspired oxygen (0.209), \(P_b\) is the measured barometric pressure (mmHg), and \(P_{H_2O}\) is the water vapor pressure of saturated air in the alveoli. \(P_{CO_2}\) (mmHg) was calculated as 
\[
4.58 \exp \left[ \frac{(17.27T_b)(237.3 + T_b)}{2} \right]
\]
where \(T_b\) is the body temperature taken as per rectum. We assumed that the partial pressure of carbon dioxide in the alveoli was equal to the \(P_{CO_2}\) (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 (SD 13) s \((n = 8)\) 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 (SD 21) s, but the subsequent degree of immobilization was not qualitatively different from 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 (SD 23) s, but zacopride coadministered 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 cardiorespiratory variables.

Respiratory rate. Etorphine administration caused a significant \((F = 3.1, P = 0.013)\) decrease in respiratory rate: before etorphine + water were injected, the respiratory rate was 27 (SD 9) breaths/min \((n = 8)\), and after etorphine + water injection, the respiratory rate decreased to 14 (SD 4) breaths/min, averaged over the 30-min immobilization period (Fig. 1). The respiratory rate returned to preinjection rates once the etorphine action was reversed with diprenorphine (Student’s paired t-test, \(P = 0.11\)). Zacopride (Student’s paired t-test, \(P = 0.91\)) and 8-OH-DPAT (Student’s paired t-test, \(P = 0.4\)) coadministered 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 compared with the etorphine + water trial.

Percent hemoglobin oxygen saturation. Etorphine administration resulted in a significant \((F = 3.1, P = 0.019)\) 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 (SD 3%) \((n = 8)\) and dropped to as low as 75 (SD 7%) after 4 min, with a gradual increase thereafter over time. After diprenorphine injection, saturation returned to near preinjection 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 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 saturation to preinjection 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 PaO\(_2\). PaO\(_2\) was 69 (SD 4) mmHg \((n = 8)\) before etorphine administration. After the injection of etorphine + water, PaO\(_2\) 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, PaO\(_2\) gradually increased, and returned to preinjection values \([F_{(10,70)} = 5.66, P = 0.5]\) after diprenorphine administration. Zacopride and 8-OH-DPAT attenuated, but did not fully abolish, the etorphine-induced decrease in PaO\(_2\), and even though the PaO\(_2\) 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 PaO\(_2\) 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 PaO\(_2\) values to preinjection values after diprenorphine administration.

Partial pressure of carbon dioxide. Administration of etorphine resulted in a significant \([F_{(10,70)} = 3.87, P < 0.05]\) increase in PaCO\(_2\), throughout the immobilization period (Fig. 4). PaCO\(_2\) was 31 (SD 2) mmHg \((n = 8)\) before etorphine administration. The highest PaCO\(_2\) value (41 (SD 5) mmHg) occurred 6 min after the etorphine + water injection and gradually decreased over time, returning to preinjection values after diprenorphine injection \([F_{(10,70)} = 3.87, P = 0.94]\). Coadministration of 8-OH-DPAT with etorphine had no beneficial effect, and the PaCO\(_2\) levels remained significantly \([F_{(10,70)} = 3.87, P < 0.001]\) elevated throughout the immobilization. Zacopride coadministration significantly attenuated the rise in PaCO\(_2\) caused by etorphine. The PaCO\(_2\) value for etorphine + zacopride was significantly \([F_{(10,70)} = 3.87, P < 0.05]\) lower than those 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 PaCO\(_2\) values to preinjection values after diprenorphine administration, whereas in the etorphine + 8-OH-DPAT trial, PaCO\(_2\) values did not return to preinjection values and remained moderately elevated \([F_{(10,70)} = 3.87, P < 0.05]\).

A-a gradient. Figure 5 shows the effect of etorphine administration, with and without coadministration of the serotonergic ligands, on a derived variable, namely, the A-a gradient in the partial pressures of oxygen. The gradient was 21 (SD 3) mmHg \((n = 8)\) before administration of etorphine. When etorphine + water were 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. Coadministration of 8-OH-DPAT with etorphine abolished the increase in the gradient \([F_{(10,70)} = 8.23, P = 0.5]\), and indeed,
the administration of etorphine was 108 (SD 12) mmHg ($n = 8$). Etorphine administration had a biphasic effect on the mean arterial pressure. For the first 6 min after etorphine + water administration, mean arterial pressure increased, and then it gradually decreased throughout the immobilization period. Coadministration of 8-OH-DPAT with etorphine enhanced the biphasic pressure changes. In the first 10-min interval, mean arterial pressure after coadministration of 8-OH-DPAT with etorphine was significantly ($F = 0.94, P = 0.0015$) higher than that following etorphine + water and etorphine + zacopride. Zacopride coadministration attenuated the biphasic effects of etorphine administration and significantly (Student’s paired $t$-test, $P = 0.025$) reduced mean arterial pressure throughout the immobilization. After the administration of diprenorphine, mean arterial pressures were significantly higher than preinjection 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, mean arterial pressure was significantly (Student’s paired $t$-test, $P = 0.007$) lower than preinjection pressure 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 one-half 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 $\text{PaO}_2 < 60$ mmHg and percent arterial hemoglobin saturation...
<85% (8). Hypoxia resulted from both a decrease in the ventilation, indicated by an increase in $\mathrm{PaCO}_2$, and a decrease in diffusion, presumably via a ventilation-perfusion mismatch, indicated by an increase in the A-a gradient in oxygen partial pressure. After 10 min, there was a gradual increase in both the $\mathrm{PaO}_2$ and the percent oxygen hemoglobin saturation values, 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. Coadministration of zacopride or 8-OH-DPAT with etorphine improved the respiratory function of the goats such that $\mathrm{PaO}_2$ and arterial hemoglobin oxygen saturation remained above levels defining clinical hypoxia. The goats experienced 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, coadministration 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 from that at sea level; $\mathrm{PaO}_2$, 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, although 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 that have investigated the function of specific 5-HT receptor 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 that 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 anesthetized 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-HT$_7$ 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-HT$_7$ receptors and not, as Sahibzada et al. had believed, on 5-HT$_{1A}$ receptors. Even if the action of 8-OH-DPAT is mediated by the 5-HT$_7$ receptors, 5-HT$_{1A}$ receptors also are facilitatory in reversing morphine-induced suppression of respiratory neurons, because buspirone, a 5-HT$_{1A}$ agonist that has no effect on the 5-HT$_7$ 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-HT$_{1A}$ and 5-HT$_7$ 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 cAMP 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, although Lalley et al. (22) found that, in anesthetized 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), 8-OH-DPAT increased the frequency of phrenic nerve discharges in anesthetized cats, but higher doses (50 and 90 μg/kg) suppressed phrenic nerve discharges. In a similar and more recent study (34), phrenic nerve discharges were decreased even when 20 μg/kg 8-OH-DPAT was injected intravenously in cats. We used a much higher dose (500 μg/kg) 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.

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-HT3a receptor isoform (28), and Manzke et al. (25) discovered that inspiratory neurons in the pre-Bötzinger complex host both 5-HT4a 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-HT4a 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 coadministered with etorphine. Nevertheless, 8-OH-DPAT coadministered did improve PaO2. We believe that this increase in 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 venu- dilation 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 serotonergic 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. (25) 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 coadministered 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 μ-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 which 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, by improving both ventilation and 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 on 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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