MAC-sparing effect of nitrous oxide in sevoflurane anesthetized sheep and its reversal with systemic atipamezole administration

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Abstract

Introduction
Nitrous oxide (N\textsubscript{2}O) is an anesthetic gas with antinociceptive properties and reduces the minimum alveolar concentration (MAC) for volatile anesthetic agents, potentially through mechanisms involving central alpha\textsubscript{2}-adrenoceptors. We hypothesized that 70% N\textsubscript{2}O in the inspired gas will significantly reduce the MAC of sevoflurane (MAC\textsubscript{SEVO}) in sheep, and that this effect can be reversed by systemic atipamezole.

Materials and methods
Animals were initially anesthetized with SEVO in oxygen (O\textsubscript{2}) and exposed to an electrical current as supramaximal noxious stimulus in order to determine MAC\textsubscript{SEVO} (in duplicates). Thereafter, 70% N\textsubscript{2}O was added to the inspired gas and the MAC re-determined in the presence of N\textsubscript{2}O (MAC\textsubscript{SN}). A subgroup of sheep were anesthetized a second time with SEVO/N\textsubscript{2}O for re-determination of MAC\textsubscript{SN}, after which atipamezole (0.2 mg kg\textsuperscript{-1}, IV) was administered for MAC\textsubscript{SNA} determinations. Sheep were anesthetized a third time, initially with only SEVO/O\textsubscript{2} to re-determine MAC\textsubscript{SEVO}, after which atipamezole (0.2 mg kg\textsuperscript{-1}, IV) was administered for determination of MAC\textsubscript{SA}.

Results
MAC\textsubscript{SEVO} was 2.7 (0.3)% [mean (standard deviation)]. Addition of N\textsubscript{2}O resulted in a 37% reduction of MAC\textsubscript{SEVO} to MAC\textsubscript{SN} of 1.7 (0.2)% (p <0.0001). Atipamezole reversed this effect, producing a MAC\textsubscript{SNA} of 3.1 (0.7)%, which did not differ from MAC\textsubscript{SEVO} (p = 0.12).
MACSEVO did not differ from MACSA (p = 0.69). Cardiorespiratory variables were not different among experimental groups except a lower ET\textsubscript{CO}2 in animals exposed to SEVO/N\textsubscript{2}O.

Conclusions
N\textsubscript{2}O produces significant MAC\textsubscript{SEVO}-reduction in sheep; this effect is completely reversed by IV atipamezole confirming the involvement of alpha\textsubscript{2}-adrenoreceptors in the MAC-sparing action of N\textsubscript{2}O.

Introduction
Nitrous oxide (N\textsubscript{2}O) is a commonly used gas anesthetic in human medicine but due to its lower efficacy in animal species [1] is currently sparingly utilized in veterinary anesthesia. Why N\textsubscript{2}O exhibits greater potency in humans than animals remains unknown [1–4]. Currently, the mechanism of action of the anesthetic and analgesic effects of N\textsubscript{2}O remains incompletely defined. With regard to its antinociceptive/analgesic actions, studies suggest an involvement of alpha\textsubscript{2}-adrenoceptors at the spinal level [5–7]. Therefore, incorporation of N\textsubscript{2}O into a balanced anesthetic protocol may help reduce the amount of volatile anesthetic required [8–10], and provide pronounced antinociception.

The current study was performed to determine the impact of N\textsubscript{2}O on the MAC of sevoflurane (SEVO) in anesthetized sheep. We hypothesized that 70% N\textsubscript{2}O, when combined with the volatile agent, will markedly reduce MAC\textsubscript{SEVO} and that this effect is mediated by activation of alpha\textsubscript{2}-adrenoceptors within the spinal cord and possibly elsewhere in the central nervous system. We further speculated that administration of atipamezole, the currently most selective, competitive alpha\textsubscript{2}-adrenoceptor antagonist, would reverse the MAC-sparing effect.

Materials and methods
Fourteen systemically healthy, ASA status I, female Sardinian sheep (2–8 years old) were included. The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Sassari (CIBASA; protocol number 22859) according to Italian legislation and was conducted in compliance with the NIH Guide for the Care of Laboratory Animals in its current form and Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes as adopted on 22 September 2010.

Pre-experimental animal preparation
Food was withheld from all sheep for 12 h prior to anesthesia, but free access to water was allowed until one h before beginning the experimental procedure. Pre-anesthetic body weight (kg), heart rate (HR, min\textsuperscript{-1}), respiratory rate (RR, min\textsuperscript{-1}), and rectal temperature (°C) were recorded. The sheep were positioned in lateral recumbency. The rostral auricular artery was catheterized after the area was clipped, aseptically prepared, and locally infiltrated with subcutaneous lidocaine 2%. The area over the lateral saphenous vein was similarly prepared and the vein catheterized.

No premedication was administered. The sheep were pre-oxygenated for 3 min with oxygen (O\textsubscript{2}) at 3 L min\textsuperscript{-1} via a tight-fitting face mask connected to a standard rebreathing system, which was attached to a workstation (Perseus\textsuperscript{®}, Dräger, Lübeck, Germany) that operates with a ventilator rebreathing system (Ventilator TurboVent\textsuperscript{2}®; Dräger, Lübeck, Germany) and
sodalime (Drägersorb®, Dräger, Lübeck, Germany) as CO₂ absorbent. During this time, baseline measurements included HR, invasive systolic (SAP), mean (MAP), and diastolic (DAP) arterial blood pressures (mmHg), arterial oxygenation (SPO₂, %) and the electrocardiogram (ECG). Saline 0.9% was administered at a rate of 5 mL kg⁻¹ h⁻¹.

Anesthesia was induced with SEVO in O₂ delivered at a vaporizer dial setting of 8% via a face mask for at least 3 min. When an adequate depth of anesthesia was achieved, determined by a lack of palpebral reflex, decreased jaw tone, and cessation of swallowing, the sheep were intubated with a 9.0-mm (ID) Murphy cuffed endotracheal tube, subsequently connected to the anesthetic circuit. Mechanical ventilation was initiated and re-adjusted to achieve normocapnea (PaCO₂, 5.3–6.6 kPa). For this purpose, arterial blood was collected in a heparin coated syringe and immediately examined using a cartridge-based blood gas analyzer (ABL 80 CO-OX flex®, Radiometer, Copenhagen, Denmark).

The HR, ECG, invasive arterial blood pressures, SpO₂, and esophageal body temperature (T, °C) were continuously monitored using a multiparameter monitor (Infinity Delta® XL, Dräger, Lübeck, Germany). The RR, tidal volume (VT, mL), fresh gas (O₂/N₂) rate (constantly 3 L min⁻¹) were monitored using the anesthesia workstation (Perseus A500®, Dräger, Lübeck, Germany) screen. The inspired fraction of O₂ and N₂O (FiO₂, FiN₂O), end-tidal sevoflurane concentration (ET<sub>SEVO</sub>, %) and end-tidal partial pressure of CO₂ (ET<sub>CO₂</sub>, kPa) were continuously monitored with the integrated multi-gas module SCIO (Dräger, Lübeck, Germany) operating as a side stream analyzer (250 mL min⁻¹), which was calibrated prior to each experiment using gas standards (1% SEVO, 5% CO₂; calibration gas, Air Liquide Healthcare America, Plumsteadville, Pennsylvania, USA).

To deliver the noxious stimulus, disposable low-resistance silver/silver chloride electrodes with an inter-electrode distance of 1 cm (Norotrode 20 Bipolar SEMG Electrodes, Myotronics-Noromed, Inc., WA, USA) were applied on the lateral metacarpal surface midway between the carpus and metacarpophalangeal joints and secured with auto-adhesive wrap (Vetrap™; St. Paul, Minnesota, USA). The electrodes were connected to a 220-volt powered constant current stimulator, model DS7A (Digitimer Ltd., Letchworth Garden City, UK) that delivered electrical impulses when triggered by a 9-volt battery powered train/delay generator, model DG2A (Digitimer Ltd., Letchworth Garden City, UK). Trains of square-wave impulses (1 ms in duration; 5 Hz, 50 mA constant current) were delivered for 60 s or until a gross purposeful motor response was noted, after which the stimulator was switched off. As the maximum delivered voltage was 200 V, the electrical resistance between electrodes was measured using an ohmmeter (Personal 20; Mega Elettronica, Milan, Italy) prior to each stimulation to ensure resistance remained at < 3 kΩ necessary to discharge a current of 50 mA. If resistance had increased to > 3 kΩ, electrodes were exchanged.

**Determination of MAC<sub>SEVO</sub> and MAC<sub>SEVO</sub> with N₂O (MAC<sub>SN</sub>)**

A modified protocol of “up-down” method as proposed by Dixon was employed [11]. After a 30-min equilibration period at an ET<sub>SEVO</sub> of 2.7%, electrical stimulation was applied, and sheep were examined for gross purposeful movement. A response was positive when major motor activity was observed in non-stimulated body parts, such as flexion or extension of the contralateral hind limb or gross neck movements. Muscle tremors, occasional swallowing, nystagmus and changes in cardio-respiratory parameters did not represent a positive response. Depending on the presence or lack of a positive response, the vaporizer dial setting was increased or decreased, respectively, to arrive at an ET<sub>SEVO</sub> 0.2% higher or lower than previously recorded. The new ET<sub>SEVO</sub> concentration was maintained for 15 min before the electrical stimulation was repeated.
The MAC value was calculated as the arithmetic mean of two ET\textsubscript{SEVO} concentrations: the value measured when the response had been positive, and the subsequent higher value recorded when the response had been negative. Each determination of MAC\textsubscript{SEVO} was performed in duplicate. After MAC\textsubscript{SEVO} determination was completed, 70% N\textsubscript{2}O was added to the inspired gas maintaining an overall fresh gas flow rate of 3 L min\textsuperscript{-1}. After a 30-min equilibration, the procedure was repeated following the protocol described above, for duplicate MAC\textsubscript{SN} determination.

**Determination of MAC\textsubscript{SN} and MAC\textsubscript{SEVO} with N\textsubscript{2}O in presence of atipamezole (MAC\textsubscript{SNA})**

After a 10-d washout period, six sheep were re-anesthetized using the tight-fitting face mask as described above. However, the anesthesia machine was now set to deliver a total fresh gas flow of 3 L min\textsuperscript{-1} with an inspired concentration of N\textsubscript{2}O of 70% (FiN\textsubscript{2}O = 0.70) in the carrier gas, with the FiO\textsubscript{2} automatically calculated and adjusted by the anesthesia workstation based on the set FiN\textsubscript{2}O. During induction of anesthesia the SEVO vaporizer dial setting was 8%. The carrier gas mixture including 70% N\textsubscript{2}O was continuously delivered at a rate of 3 L min\textsuperscript{-1} throughout the experimental procedure. After a 30-min equilibration at an ET\textsubscript{SEVO} corresponding to each animal’s previously determined MAC\textsubscript{SN}, the MAC\textsubscript{SN} was re-determined in duplicate as described. Subsequently, atipamezole (0.2 mg kg\textsuperscript{-1}) was administered IV following the pharmacodynamic and pharmacokinetic profile of this antagonist described in sheep [12]. If the MAC\textsubscript{SNA} determinations extended past 120 min of anesthesia, an additional atipamezole bolus was administered (0.1 mg kg\textsuperscript{-1}) [12]. The ET\textsubscript{SEVO} concentration was adjusted to a concentration corresponding with the individual’s MAC\textsubscript{SEVO}. Sheep were equilibrated at this ET\textsubscript{SEVO} concentration for 15 min before duplicate determination of MAC\textsubscript{SNA}.

**Determination of MAC\textsubscript{SEVO} in the presence of atipamezole (MAC\textsubscript{SA})**

Twelve months after completing the MAC\textsubscript{SNA} experiments, six of the original 12 sheep in the MAC\textsubscript{SEVO} group were re-anesthetized to determine the effect of IV atipamezole on MAC\textsubscript{SEVO}.

| Variables | No. of animals | 1\textsuperscript{st} value | 2\textsuperscript{nd} value | p-value |
|-----------|---------------|----------------------------|-----------------------------|--------|
| MAC\textsubscript{SEVO} | 12 | 2.7 (0.3)\textsuperscript{§§} | 2.7 (0.3)\textsuperscript{§§} | 0.85 |
| MAC\textsubscript{SN} | 10 | 1.7 (0.3)\textsuperscript{**} | 1.7 (0.2)\textsuperscript{**} | 0.26 |
| MAC\textsubscript{SNA} | 6 | 3.2 (0.7)\textsuperscript{§§} | 2.9 (0.8)\textsuperscript{§§} | 0.14 |
| MAC\textsubscript{SA} | 6 | 2.7 (0.6)\textsuperscript{§§} | 2.4 (0.5)\textsuperscript{§§} | 0.12 |

Presented are the mean (SD) values for first and second MAC determinations in the various experimental groups (see text for more details). The p-value in the far-right column refers to a comparison of first and second determinations of each of the MAC data presented.

Statistically significant difference from MAC\textsubscript{SEVO}

\textsuperscript{p}<0.05

\textsuperscript{**} p<0.01

Statistically significant difference from MAC\textsubscript{SN}

\textsuperscript{p}<0.05

\textsuperscript{§§} p<0.01

Statistically significant difference from MAC\textsubscript{SNA}

\textsuperscript{p}<0.05

\textsuperscript{**} p<0.01.

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The same protocol as described above for the determination of MAC$_{SEVO}$ was followed. After completion of MAC$_{SEVO}$ re-determination, IV atipamezole was administered (0.2 mg kg$^{-1}$) and re-dosed as described above. MAC$_{SA}$ determination was performed in duplicate.

**Statistical analysis**

An ad-hoc electronic dataset (Excel, Microsoft Office) was prepared to collect all data. Values for those variables determined at distinct time points during the experiments are presented in Tables 1 and 2. All MAC determinations were pooled for each animal. All quantitative data were tested for normality using a Shapiro-Wilk test. For normally distributed data, means and standard deviations (SD) are presented to describe variables pre-anesthesia and after exposure to the anesthetic agents. Non-parametric data are reported as median (IQR). Student’s t test for dependent data was employed to detect any statistically significant differences. A two-tailed p-value $<0.05$ was considered as indicating statistically significant differences between data groups. All computations were performed with STATA version 13 (Stata Corp, College Station, Texas, USA) statistical software.

**Results**

The mean (SD) age of the 14 sheep included in the MAC$_{SEVO}$, MAC$_{SN}$, and MAC$_{SNA}$ groups was 5.9 (2.0) years and 7.0 (2.6) years in the six sheep in the MAC$_{SA}$ group ($p = 0.39$). The mean weight of the sheep was 39.4 (4.4) kg and 41.5 (4.3) kg, at each time point, respectively ($p = 0.22$). Intubation required a median of 1 (1,2) attempt after 6.9 (4.0) min of SEVO inhalation.

MAC$_{SEVO}$ was determined in 12 of 14 sheep. One sheep was eliminated because an abnormal larynx anatomy prevented a regular intubation technique. A second sheep was eliminated before MAC$_{SEVO}$ determination due to a vigorous response to the first electrical stimulation that required an IV rescue anesthetic to avoid harm to the animal. The MAC$_{SN}$ was determined in 10 of 12 sheep of the MAC$_{SEVO}$ group. Two sheep demonstrated, in the presence of N$_2$O, persistent gross motor activity in response to noxious stimulation that did not, as usual, cease shortly after discontinuing stimulation but persisted for $>45$ min despite increasing ET$_{SEVO}$ to $>5.0%$. The MAC$_{SNA}$ was measured in eight sheep, although only successfully determined in six sheep. Two sheep were removed from the MAC$_{SNA}$ determination experiments due to a similar onset of persistent motor activity after noxious stimulation, either before or after atipamezole administration.

Repetitive electrical stimulation produced no tissue injury.

All MAC determinations are summarized in Table 1. The MAC$_{SEVO}$ in the present animal group was 2.7 (0.3) %, while the mean MAC$_{SN}$ was 1.7 (0.3) %, approximately 37% lower than MAC$_{SEVO}$ ($p < 0.0001$). After IV administration of atipamezole, the MAC$_{SNA}$ was 3.1 (0.7) %, which was not statistically different from MAC$_{SEVO}$ ($p = 0.12$). The negative control group, MAC$_{SA}$, did not differ from MAC$_{SEVO}$ ($p = 0.69$). As the statistical analysis of first and second MAC values in each study group (see Table 1) revealed, the first and second MAC determinations did not differ significantly from each other. Furthermore, MAC$_{SEVO}$ determinations obtained in the same sheep (Group MAC$_{SA}$) one year later were not different from originally obtained values ($p = 0.55$), justifying inclusion of those data into the MAC$_{SEVO}$ data pool.

Table 2 lists the ET$_{SEVO}$ and ETCO$_2$ and physiological variables recorded in all study groups. When compared to anesthesia with SEVO alone, SEVO in combination with N$_2$O and atipamezole, or SEVO in combination with atipamezole, exposure to SEVO/N$_2$O produced a significant increase in HR, SAP, MAP, and DAP. The ETCO$_2$ decreased significantly during SEVO/N$_2$O exposure. The SPO$_2$ during SEVO/N$_2$O anesthesia was significantly lower than
SEVO/O2 experiments, but did not produce clinically relevant hypoxemia. Other variables did not differ among any of the study groups.

The MACSEVO and MACSN, MACSNA, and MACSA determinations were completed within 405 (106), 387 (42), and 342 (62) min, respectively. The N2O exposure in the MACSN and MACSNA groups lasted 210 (85) and 387 (42) min, respectively. All sheep recovered uneventfully from anesthesia. No erythema or swelling of the electrical stimulation sites were observed. The sheep were extubated within 8.4 (5.1) min and stood within 28.6 (22.4) min after discontinuing SEVO administration.

Discussion

Results of the present study support our key hypotheses that N2O exhibits a significant MACSEVO-sparing effect in sheep and that this effect involves activation of alpha2-adrenoceptors within the central nervous system and can be reversed by administration of the selective alpha2-adrenoceptor antagonist atipamezole. The MACSEVO in this sheep population was 2.7%. The mean MACSEVO value determined in present experiments is about 40% higher than the one previously reported in three non-pregnant sheep, 1.92 (0.17%) [13], and about 18% lower than the value previously reported in six other sheep, 3.3% [14]. Such variability in MAC values is common and has been observed for various volatile agents including SEVO in many animal species [15]. Biological variation and methodological differences may account for such a discrepancy. Our MACSEVO data also compare favorably with data in goats, 2.33 (0.15) % [16].

This is the first study in sheep demonstrating a marked, i.e. 37%, reduction of MACSEVO by 70% N2O added to the inhalant gas mixture. This finding is consistent with prior studies examining the MAC-sparing effect of N2O in other animal models and for different volatile anesthetic agents. In dogs, addition of 70% N2O decreased the MAC of isoflurane by 32% [10] and

### Table 2. End-tidal concentrations of SEVO and CO2 and physiological variables recorded during MAC determinations.

| Variables                        | Baseline | Exposure to SEVO only | Exposure to SEVO plus N2O | Exposure to SEVO plus N2O plus atipamezole | Exposure to SEVO plus atipamezole | p-value | p-value** | p-value*** | p-value**** |
|----------------------------------|----------|-----------------------|---------------------------|--------------------------------------------|----------------------------------|--------|----------|----------|------------|
| End-tidal concentration of SEVO (%) | -        | 2.7 (0.4)             | 1.8 (0.4)                 | 3.2 (0.7)                                   | 2.6 (0.5)                        | <0.0001| 0.12     | 0.001    | 0.08       |
| Heart rate (min⁻¹)               | 108.0    | 97.3 (17.2)           | 117.7 (16.9)              | 107.5 (10.2)                               | 117.3 (25.0)                     | <0.0001| 0.02     | 0.11     | 0.0007     |
| Systolic arterial pressure (mmHg) | 123.0    | 114.0 (9.9)           | 124.1 (11.5)              | 108.3 (19.2)                               | 117.8 (16.3)                     | 0.008  | 0.49     | 0.02     | 0.56       |
| Mean arterial pressure (mmHg)    | 105.3    | 94.8 (11.6)           | 106.3 (10.1)              | 92.1 (18.4)                                | 99.8 (14.5)                      | 0.003  | 0.55     | 0.01     | 0.30       |
| Diastolic arterial pressure (mmHg)| 92.1 (6.9)| 84.7 (11.5)          | 95.0 (9.1)                 | 80.4 (18.3)                                | 88.1 (13.7)                      | 0.007  | 0.34     | 0.01     | 0.49       |
| Temperature (°C)                 | 39.4 (0.6)| 38.6 (0.8)            | 38.5 (0.8)                 | 37.8 (1.3)                                 | 38.1 (0.5)                      | 0.53   | 0.45     | 0.38     | 0.02       |
| End-tidal CO2 (kPa)              | -        | 5.26 (0.44)           | 4.84 (0.64)                | 4.97 (0.99)                                | 5.11 (0.36)                      | 0.05   | 0.17     | 0.74     | 0.67       |
| SPO2 (%)                         | -        | 99.3 (1.0)            | 92.1 (2.9)                 | 92.7 (4.4)                                 | 98.9 (1.3)                      | <0.0001| 0.003    | 0.84     | 0.29       |

Data represent the mean (SD) of values recorded at the time of each MAC determination, when no responses to supramaximal stimulation were recognized and average ETSEVO was at the level indicated in the first row.

*: Comparison between mean values measured during exposure to SEVO only versus exposure to SEVO plus N2O.

**: Comparison between mean values measured during exposure to SEVO only versus exposure to SEVO plus N2O plus atipamezole.

***: Comparison between mean values during exposure to SEVO plus N2O versus exposure to SEVO plus N2O plus atipamezole.

****: Comparison between mean values during exposure to SEVO only versus exposure to SEVO plus atipamezole.
of desflurane by 16% [17]; and Steffey and co-workers [1] reported with 70% N₂O an approximately 34, 31, and 37% MAC-reduction of halothane in dogs, cats, and stump-tail monkeys, respectively. The commonly reported 30–40% MAC-sparing effect of 60–75% N₂O in animal species might be considered a modest reduction compared to the up to 75% decrease in MAC of volatile agents in human patients [8,9,18]. Yet, it does compare favorably to the MAC-sparing effects of other agents, such as lidocaine, various opioids, or (dex-)medetomidine commonly used as adjuncts in balanced anesthesia regimens in clinical veterinary practice [19].

Insofar N₂O deserves consideration in both veterinary clinical practice and the laboratory animal setting. This applies particularly when the clinical condition of an animal requires limited dosing of other MAC-sparing analgesic agents and/or does not allow for increasing ET concentrations of a volatile agent. Also in moments of severe noxious stimulation during surgery, N₂O may provide fast-onset/fast-offset antinociception thanks to its favorable pharmacokinetic properties with minimal compromise in cardiorespiratory stability.

Currently the mechanisms of action of N₂O as an anesthetic remain incompletely characterized. However, it is a long-standing theory that N₂O’s anesthetic action is primarily mediated through non-competitive inhibition of the NMDA subtype of glutamate receptors in the brain, although opening of potassium channels with subsequent hyperpolarization of cerebral neurons and decreased neuronal firing may also be involved [20–21]. While still undetermined to what extent those molecular mechanisms contribute to N₂O’s MAC-sparing effects, our finding of a complete reversal of the MAC_SEVO-sparing effect of N₂O by IV administration of atipamezole supports the idea that much, if not all, of N₂O’s MAC-reducing effect is the result of enhanced alpha₂-adrenoceptor activity, and therefore potentially from antinociceptive actions. Those complex mechanisms were summarized in a comprehensive review [21], according to which, N₂O promotes the release of corticotrophin-releasing factor (CRF) from the hypothalamus, which results in increased release of endogenous opioid peptides in the periaqueductal grey in the midbrain. These opioid peptides inhibit the activity of GABA-ergic interneurons in the pons. As a result, descending noradrenergic pathways become more active and release more noradrenaline from their terminals in the spinal cord dorsal horn. There, noradrenaline stimulates alpha₁-adrenoceptors on GABA-ergic interneurons and alpha₂-receptors located post-synaptically on the second-order nociceptive neurons. This eventually leads to inhibition of nociceptive impulse conduction to supraspinal centers and thus reduced nociception/pain perception. Insofar, N₂O seems to act similarly to alpha₂-agonists such as (dex-) medetomidine and clonidine in the spinal cord, and therefore it may not be surprising that atipamezole could completely reverse its MAC-sparing action.

The determination of MAC_SA, which did not significantly differ from MAC_SEVO, shown in Table 1, indicates that atipamezole exhibits no MAC_SEVO altering action and that descending noradrenergic pathways are not tonically active and thus do not constantly suppress afferent nociceptive impulse traffic. Our observation coincides with results obtained in previous studies in dogs [22–23]. Consequently, atipamezole seems to only reverse alpha-receptor-dependent antinociceptive/analgesic mechanisms elicited by N₂O within the spinal cord. A rat study appears to further support this view because only intrathecal, but not intracerebroventricular, atipamezole administration reversed any antinociceptive effect of N₂O [5].

The majority of sheep in this study showed the typical increased motor activity with noxious stimulation that ceased within min following stimulation and with increase in ET_SEVO concentration. However, a puzzling finding in current experiments was that four of the 14 sheep enrolled (29%) demonstrated signs of long-lasting central nervous excitement in response to supramaximal noxious stimulation under SEVO/N₂O, which required their removal from the study group. The observed excitatory phenomena included gross paddling of multiple limbs, rapid nystagmus, regurgitation, and fasciculation of the neck and leg
musculature that persisted for >45 min and could not be stopped by increasing ET_{SEVO} to >5%. Undoubtedly, the noxious stimulus triggered such excitatory phenomena but no pattern was detectable that allowed prediction of such a response. Three sheep responded in such a manner to the first or second noxious stimulation under SEVO/N_{2}O anesthesia, despite not displaying excitement during preceding periods of noxious stimulation under SEVO/O_{2} anesthesia. One animal showed excitatory reactions to electrical stimulation only after the administration of atipamezole during an episode of SEVO/N_{2}O anesthesia, while not having shown central excitement in a preceding anesthetic under SEVO/N_{2}O. Despite demonstrating exaggerated excitation, all four sheep recovered uneventfully, as the other sheep. Since the phenomenon of central agitation post-noxious stimulation was only noticeable in sheep breathing N_{2}O, one may speculate whether inadequate suppression of cerebral neuronal activity promoted an unusually strong response of certain brain centers to impulses that arrived at supraspinal sites. While N_{2}O might exhibit strong antinociceptive activity at the spinal cord level that is alpha_{2}-adrenoceptor mediated, its depressant (i.e. hypnotic/anesthetic) effect at the cerebral level might be weaker, non-uniform, or different in sheep than in other species. Unfortunately, MACN_{2}O data have not been reported in sheep but values in other animal species and the human reveal significant inter-species differences. For example, for dogs, cats, pigs, calves, and humans MACN_{2}O values of average 236, 255, 211, 223, and 104%, respectively are reported [15].

Interestingly, electroencephalography (EEG) studies in humans indicate that N_{2}O can significantly diminish the depressant effects of volatile anesthetics such as isoflurane [24] and sevoflurane [25] on certain cerebral neuronal activity, thereby acting centrally in opposition to the volatile anesthetics. In SEVO-anesthetized human patients, addition of 60% N_{2}O produced an 11% increase in the ED_{50} of SEVO for induction of an isoelectric EEG (p < 0.0001), while it did not significantly affect the ED_{50} for electroencephalographic burst suppression [25]. It will require a detailed EEG analysis to potentially elucidate the mechanisms behind the central arousal phenomenon and motor activities observed in some of the SEVO/N_{2}O anesthetized sheep in this study.

A limitation for the interpretation of present pulse oximeter data is that no arterial blood gas samples were collected throughout the different phases of the study to more objectively assess the arterial oxygenation status in each animal, particularly when breathing a gas mixture containing 70% N_{2}O. In preceding pilot experiments, arterial blood gases had been measured and during the exposure of 70% N_{2}O, the lowest PaO_{2} determined was 68 mmHg, which is associated with an arterial oxygen saturation of >90% and thus within a clinically acceptable range given the reduced FiO_{2}. We recorded only during the second MAC_{SNA} determination in two sheep temporarily an SpO_{2} of <90% (minimum 83%). Without corresponding arterial blood gas data available, it is difficult to predict the accuracy of those pulse oximeter readings. Nonetheless, those readings do emphasize the need for repetitive arterial blood gas analyses when gas mixtures relatively low in FiO_{2} are used in clinical practice and to increase the FiO_{2} in case arterial oxygen saturation decreases below normal to avoid persistent hypoxemia.

In conclusion, this study demonstrates that N_{2}O exerts a significant MAC-reducing effect in sheep. Systemic administration of atipamezole reverses the MAC reduction, supporting the notion that alpha_{2}-adrenoceptor dependent mechanisms in the spinal cord dorsal horn and possibly also in the brain are involved in this activity.

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