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

Lauren Duffee[1]☯*, Nicolò Columbano[2,3]☯, Antonio Scanu[2‡], Valentino Melosu[2], Giovanni Mario Careddu[2,3], Giovanni Sotgiu[4‡], Bernd Driessen[1,5]☯

1 Department of Clinical Studies-New Bolton Center, University of Pennsylvania, School of Veterinary Medicine, Kennett Square, Pennsylvania, United States of America, 2 Dipartimento di Medicina Veterinaria, Università degli Studi di Sassari, Sassari, Sardegna, Italy, 3 Centro di Ricerca di Chirurgia Comparata (CRCC), Università degli Studi di Sassari, Sassari, Sardegna, Italy, 4 Dipartimento di Scienze Biomediche, Università degli Studi di Sassari, Sassari, Sardegna, Italy, 5 Narkovet Consulting™ LLC, Chadds Ford, PA, United States of America

☯ These authors contributed equally to this work.
‡ These authors also contributed equally to this work.
* lduffee@upenn.edu

Abstract

Introduction

Nitrous oxide (N_2O) is an anesthetic gas with antinociceptive properties and reduces the minimum alveolar concentration (MAC) for volatile anesthetic agents, potentially through mechanisms involving central alpha$_2$-adrenoceptors. We hypothesized that 70% N_2O in the inspired gas will significantly reduce the MAC of sevoflurane (MAC_{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_2) and exposed to an electrical current as supramaximal noxious stimulus in order to determine MAC_{SEVO} (in duplicates). Thereafter, 70% N_2O was added to the inspired gas and the MAC re-determined in the presence of N_2O (MAC_{SN}). A subgroup of sheep were anesthetized a second time with SEVO/N_2O for re-determination of MAC_{SN}, after which atipamezole (0.2 mg kg^{-1}, IV) was administered for MAC_{SNA} determinations. Sheep were anesthetized a third time, initially with only SEVO/O_2 to re-determine MAC_{SEVO}, after which atipamezole (0.2 mg kg^{-1}, IV) was administered for determination of MAC_{SA}.

Results

MAC_{SEVO} was 2.7 (0.3)% [mean (standard deviation)]. Addition of N_2O resulted in a 37% reduction of MAC_{SEVO} to MAC_{SN} of 1.7 (0.2)% (p <0.0001). Atipamezole reversed this effect, producing a MAC_{SNA} of 3.1 (0.7)%, which did not differ from MAC_{SEVO} (p = 0.12).

MAC$_{SEVO}$ did not differ from MAC$_{SA}$ (p = 0.69). Cardiorespiratory variables were not different among experimental groups except a lower ETCO$_2$ in animals exposed to SEVO/N$_2$O.

Conclusions

N$_2$O produces significant MAC$_{SEVO}$-reduction in sheep; this effect is completely reversed by IV atipamezole confirming the involvement of alpha$_2$-adrenoreceptors in the MAC-sparing action of N$_2$O.

Introduction

Nitrous oxide (N$_2$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$_2$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$_2$O remains incompletely defined. With regard to its antinociceptive/analgesic actions, studies suggest an involvement of alpha$_2$-adrenoceptors at the spinal level [5–7]. Therefore, incorporation of N$_2$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$_2$O on the MAC of sevoflurane (SEVO) in anesthetized sheep. We hypothesized that 70% N$_2$O, when combined with the volatile agent, will markedly reduce MAC$_{SEVO}$ and that this effect is mediated by activation of alpha$_2$-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$_2$-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^{-1}), respiratory rate (RR, min^{-1}), and rectal temperature (T,˚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$_2$) at 3 L min^{-1} via a tight-fitting face mask connected to a standard rebreathing system, which was attached to a workstation (Perseus®, Dräger, Lübeck, Germany) that operates with a ventilator rebreathing system (Ventilator TurboVent2®, Dräger, Lübeck, Germany) and

sodalime (Drägersorb®, Dräger, Lübeck, Germany) as CO$_2$ absorbent. During this time, baseline measurements included HR, invasive systolic (SAP), mean (MAP), and diastolic (DAP) arterial blood pressures (mmHg), arterial oxygenation (SPO$_2$, %) 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 O$_2$ 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 (PaCO$_2$, 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, SpO$_2$, 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 (O$_2$/N$_2$O) rate (constantly 3 L min^{-1}) were monitored using the anesthesia workstation (Perseus A500®, Dräger, Lübeck, Germany) screen. The inspired fraction of O$_2$ and N$_2$O (FiO$_2$, FiN$_2$O), end-tidal sevoflurane concentration (ET$_{SEVO}$, %) and end-tidal partial pressure of CO$_2$ (ET$_{CO2}$, 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% CO$_2$; 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 TM; 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$_{SEVO}$ and MAC$_{SEVO}$ with N$_2$O (MAC$_{SN}$)

A modified protocol of "up-down" method as proposed by Dixon was employed [11]. After a 30-min equilibration period at an ET$_{SEVO}$ 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$_{SEVO}$ 0.2% higher or lower than previously recorded. The new ET$_{SEVO}$ concentration was maintained for 15 min before the electrical stimulation was repeated.

The MAC value was calculated as the arithmetic mean of two ET$_{SEVO}$ 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$_{SEVO}$ was performed in duplicate. After MAC$_{SEVO}$ determination was completed, 70% N$_2$O was added to the inspired gas maintaining an overall fresh gas flow rate of 3 L min^{-1}. After a 30-min equilibration, the procedure was repeated following the protocol described above, for duplicate MAC$_{SN}$ determination.

Determination of MAC$_{SN}$ and MAC$_{SEVO}$ with N$_2$O in presence of atipamezole (MAC$_{SNA}$)

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^{-1} with an inspired concentration of N$_2$O of 70% (FiN$_2$O = 0.70) in the carrier gas, with the FiO$_2$ automatically calculated and adjusted by the anesthesia workstation based on the set FiN$_2$O. During induction of anesthesia the SEVO vaporizer dial setting was 8%. The carrier gas mixture including 70% N$_2$O was continuously delivered at a rate of 3 L min^{-1} throughout the experimental procedure. After a 30-min equilibration at an ET$_{SEVO}$ corresponding to each animal's previously determined MAC$_{SN}$, the MAC$_{SN}$ was re-determined in duplicate as described. Subsequently, atipamezole (0.2 mg kg^{-1}) was administered IV following the pharmacodynamic and pharmacokinetic profile of this antagonist described in sheep [12]. If the MAC$_{SNA}$ determinations extended past 120 min of anesthesia, an additional atipamezole bolus was administered (0.1 mg kg^{-1}) [12]. The ET$_{SEVO}$ concentration was adjusted to a concentration corresponding with the individual's MAC$_{SEVO}$. Sheep were equilibrated at this ET$_{SEVO}$ concentration for 15 min before duplicate determination of MAC$_{SNA}$.

Determination of MAC$_{SEVO}$ in the presence of atipamezole (MAC$_{SA}$)

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

Table 1. First and second determinations of MAC$_{SEVO}$, MAC$_{SN}$, MAC$_{SNA}$, MAC$_{SA}$, and MAC$_{SEVO}$ in the form of pooled data.

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

*$p < 0.05$

** $p < 0.01$

statistically significant difference from MAC$_{SN}$

§$p < 0.05$

§§ $p < 0.01$

statistically significant difference from MAC$_{SNA}$

†$p < 0.05$

†† $p < 0.01$.

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

Table 2. End-tidal concentrations of SEVO and CO$_2$ and physiological variables recorded during MAC determinations.

Variables	Baseline	Exposure to SEVO only	Exposure to SEVO plus N$_2$O	Exposure to SEVO plus N$_2$O 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 (28.2)	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 (8.2)	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 (7.0)	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 CO$_2$ (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
SPO$_2$ (%)	-	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 ET$_{SEVO}$ was at the level indicated in the first row.

*: Comparison between mean values measured during exposure to SEVO only versus exposure to SEVO plus N$_2$O.

**: Comparison between mean values measured during exposure to SEVO only versus exposure to SEVO plus N$_2$O plus atipamezole.

***: Comparison between mean values during exposure to SEVO plus N$_2$O versus exposure to SEVO plus N$_2$O plus atipamezole.

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

SEVO/O$_2$ experiments, but did not produce clinically relevant hypoxemia. Other variables did not differ among any of the study groups.

The MAC$_{SEVO}$ and MAC$_{SN}$, MAC$_{SNA}$, and MAC$_{SA}$ determinations were completed within 405 (106), 387 (42), and 342 (62) min, respectively. The N$_2$O exposure in the MAC$_{SN}$ and MAC$_{SNA}$ 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 N$_2$O exhibits a significant MAC$_{SEVO}$-sparing effect in sheep and that this effect involves activation of alpha$_2$-adrenoceptors within the central nervous system and can be reversed by administration of the selective alpha$_2$-adrenoceptor antagonist atipamezole. The MAC$_{SEVO}$ in this sheep population was 2.7%. The mean MAC$_{SEVO}$ 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 MAC$_{SEVO}$ 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 MAC$_{SEVO}$ by 70% N$_2$O added to the inhalant gas mixture. This finding is consistent with prior studies examining the MAC-sparing effect of N$_2$O in other animal models and for different volatile anesthetic agents. In dogs, addition of 70% N$_2$O decreased the MAC of isoflurane by 32% [10] and

of desflurane by 16% [17]; and Steffey and co-workers [1] reported with 70% N$_2$O 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% N$_2$O 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 N$_2$O 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, N$_2$O 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 N$_2$O as an anesthetic remain incompletely characterized. However, it is a long-standing theory that N$_2$O'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 N$_2$O's MAC-sparing effects, our finding of a complete reversal of the MAC$_{SEVO}$-sparing effect of N$_2$O by IV administration of atipamezole supports the idea that much, if not all, of N$_2$O's MAC-reducing effect is the result of enhanced alpha$_2$-adrenoreceptor activity, and therefore potentially from antinociceptive actions. Those complex mechanisms were summarized in a comprehensive review [21], according to which, N$_2$O 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 alpha$_1$-adrenoceptors on GABA-ergic interneurons and alpha$_2$-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, N$_2$O seems to act similarly to alpha$_2$-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 N$_2$O 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 N$_2$O [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/N$_2$O, 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.

Acknowledgments

degli Studi di Sassari belongs, for integrating applied anesthesia research to the scientific scope of CRCC and making funding resources and logistical support of the center available for the conduction of the present study. We thank Dräger, Italy for having provided us with technical support throughout the study period.

Author Contributions

Conceptualization: Nicolò Columbano, Bernd Driessen.

Formal analysis: Lauren Duffee, Giovanni Sotgiu.

Funding acquisition: Nicolò Columbano, Giovanni Mario Careddu, Bernd Driessen.

Investigation: Lauren Duffee, Nicolò Columbano, Antonio Scanu, Valentino Melosu, Giovanni Mario Careddu, Bernd Driessen.

Methodology: Lauren Duffee, Nicolò Columbano, Bernd Driessen.

Resources: Nicolò Columbano, Giovanni Mario Careddu.

Writing – original draft: Lauren Duffee.

Writing – review & editing: Lauren Duffee, Nicolò Columbano, Giovanni Sotgiu, Bernd Driessen.

References

1. Steffey EP, Gillespie JR, Berry JD, Eger EI, Munson ES. Anesthetic potency (MAC) of nitrous oxide in the dog, cat, and stump-tail monkey. J Appl Physiol. 1974; 36: 530–532. https://doi.org/10.1152/jappl.1974.36.5.530 PMID: 4826313

2. Hornbein TF, Eger EI, Winter PM, Smith G, Wetstone D, Smith KH. The minimum alveolar concentration of nitrous oxide in man. Anesth Analg. 1982; 61: 553–556. PMID: 7201254

3. Russell GB, Graybeal JM. Direct measurement of nitrous oxide MAC and neurologic monitoring in rats during anesthesia under hyperbaric conditions. Anesth Analg. 1992; 75: 995–999. PMID: 1443718

4. Weiskopf RB, Bogetz MS. Minimum alveolar concentrations (MAC) of halothane and nitrous oxide in swine. Anesth Analg. 1984; 63: 529–532. PMID: 6711846

5. Guo TZ, Poree L, Golden W, Stein J, Fujinaga M, Maze M. Antinociceptive response to nitrous oxide is mediated by supraspinal opiate and spinal a2adrenergic receptors in the rat. Anesth. 1996; 85: 846–852.

6. Guo TZ, Davies FM, Kingery WS, Patterson AJ, Limbird LE, Maze M. Nitrous oxide produces antinociceptive response via a2B and/or a2C adrenoceptor subtypes in mice. Anesth. 1999; 90: 470–476.

7. Sawamura S, Kingery WS, Davies MF, Geeta SA, Clark JD, Kobilka BK, et al. Antinociceptive action of nitrous oxide is mediated by stimulation of noradrenergic neurons in the brainstem and activation of a2B adrenoceptors. J Neurosci. 2000; 20: 9242–9251. PMID: 11125002

8. Stevens WC, Dolan WM, Gibbons RT, White A, Eger EI II, Miller RD, et al. Minimum alveolar concentration (MAC) of isoflurane with and without nitrous oxide in patients of various ages. Anesth. 1975; 42: 197–200.

9. Ghouri AF, White PF. Effect of fentanyl and nitrous oxide on the desflurane anaesthetic requirement. Anesth Analg. 1991; 72: 377–381. PMID: 1994766

10. Voulgaris DA, Egger CM, Seddighi MR, Rohrbach BW, Love LC, Doherty TJ. The effect of nitrous oxide on the minimum alveolar concentration (MAC) and MAC derivatives of isoflurane in dogs. Can J Vet Res. 2013; 77: 131–135. PMID: 24082405

11. Dixon WJ, Massey FJ. The "up-and-down" method. In: Dixon WJ, Massey FJ, editors. Introduction to Statistical Analysis. 4th ed. New York: McGraw-Hill; 1983. pp. 428–439.

12. Ranheim B, Arnemo JM, Stuen S, Horsberg TE. Medetomidine and atipamezole in sheep: disposition and clinical effects. J Vet Pharmacol Ther. 2000; 23: 401–404. PMID: 11168919

13. Okutomi T, Whittington RA, Stein DJ, Morishima HO. Comparison of the effects of sevoflurane and isoflurane anesthesia on the maternal-fetal unit in sheep. J Anesth. 2009; 23: 392–398. https://doi.org/10.1007/s00540-009-0763-2 PMID: 19685120

14. Lukasik VM, Nogami WM, Morgan SE. Minimum alveolar concentration and cardiovascular effects of sevoflurane in sheep. Vet Surg. 1997; 27: 168.

15. Steffey EP, Mama KR, Brosnan RJ. Inhalation anesthetics. In: Grimm KA, Lamont LA, Tranquilli WJ, Greene SA, Robertson SA, editors. Veterinary Anesthesia and Analgesia. 5th ed. Ames: Wiley Black-well; 2015. pp. 297–331.

16. Hikasa Y, Okuyama K, Kakuta T, Takase K, Ogasawara S. Anesthetic potency and cardiopulmonary effects of sevoflurane in goats: comparison with isoflurane and halothane. Can J Vet Res. 1998; 62: 299–306. PMID: 9798097

17. Nishimori CT, Nunes N, Paula DP, Rezende ML, Souza AP, Santos PSP. Effects of nitrous oxide on minimum alveolar concentration of desflurane in dogs. Arq Bras Med Vet Zootec. 2007; 59: 97–104.

18. Saidman LJ, Eger EI. Effect of nitrous oxide and of narcotic premedication on the alveolar concentration of halothane required for anesthesia. Anesth. 1964; 25: 302–306.

19. Acevedo-Arcique CM, Ibancovichi JA, Gutierrez-Blanco E, Moran-Muñoz R, Victoria-Mora JM, Tendillo-Cortijo F, et al. Lidocaine, dexmedetomidine and their combination reduce isoflurane minimum alveolar concentration in dogs. PloS One. 2014; 9: 1–5.

20. Jevtovic-Todorovic V, Todorovc SM, Mennerick S, Powell S, Dikranian K, Benshoff N, et al. Nitrous oxide (laughing gas) is an NMDA antagonist, neuroprotectant and neurotoxin. Nat Med. 1998; 4: 460–463. PMID: 9546794

21. Sanders RD, Weimann J, Maze M. Biologic effects of nitrous oxide. Anesth. 2008; 109: 707–722.

22. Ewing KK, Mohammed HO, Scarlett JM, Short CE. Reduction of isoflurane anaesthetic requirement by medetomidine and its restoration by atipamezole in dogs. Am J Vet Res. 1993; 54: 294–299. PMID: 8094277

23. Bloor BC, Flacke WE. Reduction in halothane anaesthetic requirement by clonidine, an alpha-adrenergic agonist. Anesth Analg. 1982; 61: 741–745. PMID: 6125112

24. Yli-Hankala A, Lindgren L, Porkkala T, Jantti V. Nitrous oxide-mediated activation of the EEG during isoflurane anaesthesia in patients. Br J Anaesth. 1993; 70: 54–57. PMID: 8431334

25. Niu B, Xiao JY, Fang Y, Zhou BY, Li J, Cao F, et al. Sevoflurane-induced isoelectric EEG and burst suppression: differential and antagonistic effect of added nitrous oxide. Anesth. 2017; 72: 570–579.