Available online at www.sciencedirect.com
ScienceDirect
Respiratory Physiology & Neurobiology 156 (2007) 94–101
RESPIRATORY PHYSIOLOGY & NEUROBIOLOGY
www.elsevier.com/locate/resphysiol

Exhaled nasal nitric oxide output is reduced in humans at night during the sleep period

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Accepted 9 August 2006

Abstract

The physiologic function of nasal nitric oxide (NO) release is unknown. In prior experiments, topical N^G-nitro-L-arginine methyl ester (L-NAME) on nasal mucosa reduced exhaled nasal NO output and caused daytime sleepiness. We hypothesized that nasal NO output is reduced at night during the sleep period. We measured exhaled nasal NO concentration and minute ventilation and calculated nasal NO output in humans over 24 h. Daytime awake NO output was greater than NO output at night during sleep or transient wakefulness. Exhaled NO concentration decreased during sleep along with minute ventilation. A daytime voluntary reduction in minute ventilation also decreased nasal NO output but exhaled NO concentration increased. Nasal NO output was not changed by body position. We conclude that exhaled nasal NO output is decreased at night due to decreased mass flow of NO into nasal air in addition to decreased minute ventilation. Our findings suggest a role of nasal NO in sleep or in the physiologic processes accompanying sleep.

Keywords: Sleep; Somnolence; Nitric oxide; Nose

1. Introduction

Nitric oxide (NO) is present in the exhaled air of humans. The majority of exhaled NO is released into the nasal passages and paranasal sinuses (Lundberg et al., 1994; Kimberly et al., 1996). The function of NO in the upper airway and factors that modulate its release into nasal air are poorly understood. In prior experiments we have shown that an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME), delivered by aerosol into the nasal cavity, decreased exhaled NO and altered temperature conditioning and humidity conditioning of inhaled and exhaled air in humans (Holden et al., 1999). Unexpectedly, the subjects complained of sleepiness during and following these experiments. This observation prompted us to study the effects of L-NAME on daytime sleepiness (Sippel et al., 1999). We found that L-NAME reduced exhaled NO and shortened sleep onset latency, indicating increased daytime sleepiness. These findings suggested a potential relationship between decreased nasal exhaled NO output and increased sleepiness or sleep in humans, but the mechanism by which the topical L-NAME induced sleepiness remains unknown. Other researchers have shown that inhibition of NO synthase reduces wakefulness in rats (Dzoljic and De Vries, 1994). In addition, rat brain cortical NO may be reduced during sleep (Burlet and Cespuglio, 1997; Faradji et al., 2000). This observation led us to speculate that nasal NO output might be naturally deceased at night when humans are somnolent or asleep. We therefore hypothesized that exhaled nasal NO output is reduced at night. To test this hypothesis, we measured exhaled nasal nitric oxide output in adult humans during daytime wakefulness and nighttime sleep and wakefulness. Nighttime sleep was documented using polysomnography. Since minute ventilation is reduced in sleep compared to the awake state, and since NO output could be a function of minute ventilation alone, we also measured exhaled nasal NO output during daytime voluntary hypoventilation in a range of minute ventilation values similar to those measured during the night. Finally, in order to evaluate the potential variation in NO output due to the supine posture typical of sleep, we compared nasal NO output between the upright and supine positions.

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doi:10.1016/j.resp.2006.08.002

2. Methods

2.1. Subjects

The Investigational Review Board of the Portland VA Medical Center approved the research protocol and subjects gave their written consent before participation. Eight healthy subjects (seven males and one female, mean age 38 years, range 21–61) participated in the daytime awake, nighttime sleep portion of the study. Eleven healthy subjects (five males and six females, mean age 42 years, range 22–62) participated in the voluntary reduction in minute ventilation experiments. Nine healthy subjects (seven males, two females, mean age 48 years, range 29–63) participated in the upright versus supine position experiments.

2.2. Measurement of nasal NO output, O_2 consumption and CO_2 output

Subjects, with mouth closed, breathed into a nasal continuous positive airway pressure mask (Respironics, Inc., Murrysville, PA, USA) connected to a two-way valve (Hans–Rudolph, Inc., Kansas City, KS, USA) that allowed for inhalation of room air through one port and collection of exhaled air through the other port. The exhalation port of the valve was attached to a Mylar bag known to be impermeable to and non-reactive with NO. We measured NO concentration (ppb) using a chemiluminescence analyzer (Sievers Instruments, Inc., model 270B, Boulder, CO, USA) calibrated using precise dilutions of a certified gas mixture (45 ppm NO in air, Sievers Instruments, Inc.) in NO-free air using a calibrated two-liter syringe.

With each exhaled gas measurement, concurrent ambient levels of NO were also measured. In general, ambient NO levels are negligible in Portland, OR, at night, and were consistently found to be $\leq$5 ppb during the daytime experiments reported herein. To determine minute ventilation, the volume of each timed collection of exhaled air was measured in a Tissot gasometer. Nasal NO output (nL/min) was calculated as the product of NO concentration in the collection bag and minute ventilation:

$$\text{NO output (nL/min)} = [\text{NO}]\,(\text{ppb or nL/L}) \times \text{minute ventilation (L/min)}$$

Because we have previously noted a direct relationship between body mass and exhaled nasal NO output (unpublished observation), the results are expressed per m^2 of body surface area. Oxygen consumption and carbon dioxide output were measured to document during the awake and asleep states during the day and at night. The percentage of O_2 and of CO_2 in exhaled air was measured using a calibrated (certified gas mixtures of O_2 and CO_2 in nitrogen) mass spectrometer (MGA 100, Perkin-Elmer, Inc., Pomona, CA, USA) or metabolic cart (Sensormedics, Inc., Yorba Linda, CA, USA).

2.3. Nasal gas collections over a 24-h period

Hourly daytime measurements were made with the subjects seated and resting quietly for 2 min before exhaled nasal gas collections were collected over 2 min. Similar nighttime exhaled gas collections were made every 20 min while subjects were supine in bed during the sleep period (approximately from 11 p.m. to 6 a.m.). Sleep stages were monitored at night using surface electroencephalographic (EEG) activity of the central and occipital regions and submental electromyography. The sleep state (wake versus sleep) was determined from the EEG record using standard criteria (Rechtschaffen and Kales, 1968). While the subjects were supine in bed, a chinstrap was used during the nocturnal recordings to ensure mouth closure and nasal breathing. Subjects were monitored for oral breathing during the night by observation via video camera and oral air breathing was not observed during the nocturnal recordings.

2.4. Measurements of exhaled nasal NO output during voluntary changes in minute ventilation

Minute ventilation is reduced during sleep compared with wakefulness, primarily due to decreased tidal volume and mean inhaled airflow (Phillipson and Bowes, 1986). In a previous study we observed that decreases in airflow in a closed nasal circuit decreased nasal NO output slightly over the range of airflow that occurs with breathing (Giraud et al., 1998). To determine if changes in minute ventilation could account for the differences in nasal NO output between daytime wakefulness and nighttime sleep in the 24-h measurements, we measured exhaled nasal NO output during the day in 11 awake, seated subjects during resting normal ventilation and voluntary hypoventilation. The subjects voluntarily controlled their minute ventilation by altering respiratory rate and/or tidal volume in whatever way they found most comfortable over a range of 5–10 L/min (the range of minute ventilation observed in our 24-h sleep wake study). No attempt was made to selectively decrease tidal volume, respiratory rate or airflow rate during voluntary hypoventilation over the 2-min gas collection.

2.5. Measurements of exhaled nasal NO output with change in position

The supine posture of sleep typically differs from the upright status characteristic of daytime wakefulness. To determine the potential contribution of body position on the difference in nasal NO output between wakefulness and sleep, we measured nasal NO output during the day in nine awake subjects after a 30-min period of rest in both the seated and supine positions.

2.6. Statistical analysis

All numeric data in the text is expressed as mean with standard deviation and in the illustrations as mean with standard error of the mean. The unit of analysis was each exhaled gas collection. Linear mixed models were used to fit the data where sleep state was the fixed effect and study subject was the random effect (Neter et al., 1990). Tukey-Kramer adjustments were used for multiple comparisons with an alpha level of 0.05. Paired Student's *t*-tests were used in the analyses of ventilation state and body position data with statistical significance set at $p < 0.05$.