Increased airway osmolarity inhibits the action of nitric oxide in the rabbit

M. Högman, J. Hjoberg, G. Hedenstierna

Increased airway osmolarity inhibits the action of nitric oxide in the rabbit. M. Högman, J. Hjoberg, G. Hedenstierna. ©ERS Journals Ltd 1998.

ABSTRACT: Inhalation of nitric oxide (NO) is known to dilate preconstricted airways. In asthmatics, there are large variations in the effect of NO on airway tone. One explanation of these variations may be different degrees of airway wall oedema.

The effect of NO inhalation on methacholine (meth)-induced airway constriction was investigated in a rabbit model. Oedema and a change in osmolarity of the airways was achieved by hypertonic saline nebulization and hyperventilation with dry gas.

There was an increase in resistance to meth at a concentration of 3 mg·mL^{-1}, of 86±14 cmH$_2$O·L^{-1}·s (mean±SEM) after oedema formation, compared with 46±16 cmH$_2$O·L^{-1}·s without oedema. Inhalation of 80 parts per million (ppm) NO failed to counter the increase in resistance due to meth, 92±14 cmH$_2$O·L^{-1}·s after hypertonic saline nebulization. After hyperventilation of dry gas, the increase in resistance due to meth at 1 mg·mL^{-1} was 27±11 cmH$_2$O·L^{-1}·s with 80 ppm NO and 28±5 cmH$_2$O·L^{-1}·s without NO.

In conclusion, the relaxant effect of nitric oxide-inhalation on the airway smooth muscle can be blocked by an increase in the osmolarity of the airway surface liquid. The mechanism of this inhibition of nitric oxide remains to be established.
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Dept of Clinical Physiology, University Hospital and Asthma Research Centre, University of Uppsala, Uppsala, Sweden.

Correspondence: M. Högman, Dept of Clinical Physiology, University Hospital, S-751 85 Uppsala, Sweden, Fax: 46 18 664135

Endogenous nitric oxide modulates neural bronchoconstriction in both experimental animals [1] and humans [2]. NO is the only known neural bronchodilator of the inhibitory nonadrenergic noncholinergic nerves in human airways [3]. Inhalation of NO has been shown to reduce the response to bronchoconstricting agents in both animals [4–7] and humans [8, 9]. In a study on asthmatic patients, inhalation of NO at a concentration of 80 parts per million (ppm) reduced the airway resistance [8]; however, there were large variations. In some patients, no improvement at all was observed in airway tone after inhalation of NO, while others showed the same dilatory effect as for a β$_2$-agonist.

Asthma is an inflammatory disease, characterized by the migration of inflammatory cells into the airway wall and the formation of oedema. The increase in the osmolarity of the airway surface liquid seen during hyperventilation has been proposed as the stimulus for exercise-induced bronchoconstriction [10]. In a rabbit model, hyperventilation with dry gas causes an increased response to histamine with respect to resistance [11]. During nebulization with hypertonic saline there is a shift in the ion content of the airway wall. This shift of ions causes fluid accumulation, and hence, a transient oedema is formed [12]. It was hypothesized that the blunted response to NO inhalation in some patients with asthma is caused by an oedema or increased osmolarity of the airway wall. Therefore, the effectiveness of inhaled NO in a rabbit model in countering bronchoconstriction was investigated after nebulization of hypertonic saline; and after hyperventilation with dry gas.

In rabbits, as in humans, a concentration of Š80 ppm NO is needed to exert a relaxing effect on bronchial smooth muscle [5, 6]. In this study, concentrations of 80–300 ppm NO were used. Methacholine (meth) was used to induce bronchoconstriction and evaluate the bronchoprotective effect of the inhaled NO.

Materials and methods

New Zealand White rabbits of both sexes with a body weight of 3.0–3.7 kg were used. They were vaccinated against pasteurella and bordetella and maintained on water and 75 g high-protein pellets·day^{-1} *ad libitum*. The protocol was approved by the regional ethics committee on animal experiments.

Anaesthesia and animal preparation

Premedication was given *i.m.* with fluanisone and phentanylcitrate (Hypnorm; Janssen Pharmaceutica, Beerse, Belgium) at 0.2 mL·kg^{-1}. Neuroleptic anaesthesia (0.3 mL·kg^{-1} Hypnorm and 2 mg·kg^{-1} diazepam) was given before oral intubation with a cuffed tube of 3.0 mm diameter (Sheridan, Argyle, NY, USA). The anaesthesia was continued with *i.v.* infusion of 0.1–0.3 mL·kg^{-1}·h^{-1} Hypnorm,

and with 2.5 mg diazepam *i.v.* and 0.2 mg pancuronium bromide *i.v.* when necessary.

The marginal ear vein was used for *i.v.* injections, while the ear artery was used for blood sampling and pressure monitoring. The rabbit was placed in the prone position on a heating pad to maintain normal body temperature. Artificial ventilation was given with a Siemens 900C ventilator (Siemens-Elema, Solna, Sweden) with an inspiratory oxygen fraction (FI,O_2) of 0.3, an inspiratory:expiratory (I:E) ratio of 1:2, a tidal volume of 38 mL and a ventilatory frequency of approximately 30 breaths·min^{-1}. The ventilatory frequency was adjusted to keep the end-tidal CO_2 tension (PET,CO_2), measured with an Eliza duo (Datex-Engström, Bromma, Sweden), at around 5 kPa. After induction of anaesthesia, 30 min were allowed before the onset of the experiment. At the end of the experiment, muscle paralysis was antagonized with 0.15 mg neostigmine and 0.03 mg glycopyrron. In addition, *i.v.* naloxone was given to counteract hypoventilation due to the residual effects of Hypnorm.

Measurements

Arterial blood was drawn for the determination of arterial oxygen tension (Pa,O_2) (ABL 300; Radiometer, Copenhagen, Denmark). The ratio of the Pa,O_2 to the FI,O_2 was used as an index of arterial oxygenation. NO (AGA AB, Lidingö, Sweden) was administered, as a gas mixture with 30% oxygen in nitrogen, to the low-pressure gas supply inlet of the ventilator. A soda lime canister was fitted into the breathing circuit to eliminate any nitrogen dioxide formed. The NO concentration was monitored in the inhalation gas using a chemiluminescence technique (Model 9841; Monitor Labs, Englewood, CO, USA).

The methaemoglobin (MetHb) concentration was determined by spectrophotometry (OSM 3; Radiometer) before and after NO inhalation. Mean arterial pressure (MAP) was continuously followed (Tram 7010; Marquette Electronics, Milwaukee, WI, USA). Respiratory resistance (Rrs) and lung compliance (Crs) were obtained using the technique of inspiratory-hold manoeuvre after constant-flow inflation [13]. Pressure and flow signals were measured at the inspiratory side of the ventilator and fed into a computer for on-line signal processing by LabView 3.0 software (National Instruments, Austin, TX, USA). Volume and flow values were corrected for gas compression in the breathing circuit. Rrs is the difference between the maximum airway pressure and the pressure after a two-second end-inspiratory pause, divided by the flow. Rrs is presented with the endotracheal resistance of 28 cmH$_2$O·L^{-1}·s subtracted. Crs was calculated as the tidal volume divided by the end-inspiratory pressure minus the end-expiratory pressure.

Protocols

Each protocol was divided into one experiment with NO and one experiment without NO inhalation in the same rabbits. The sequence of the experiments was randomized. There was a resting period of Š2 weeks between experiments within a protocol. Rabbits participating in more than one protocol rested for Š2 months between protocols.

Protocol 1: effects of nitric oxide inhalation on bronchoconstriction. This protocol served as a control to test whether NO had an effect on induced bronchoconstriction in the absence of airway wall oedema, as in earlier studies [5, 6]. Eight rabbits were studied. NO was inhaled at a concentration of 80 ppm for 10 min before and also during the meth challenge. Meth was nebulized at a concentration of 3 mg·mL^{-1}.

Protocol 2: effects of nitric oxide inhalation on bronchoconstriction after nebulization of hypertonic saline. Nebulization of phosphate-buffered hypertonic (3.6%) saline, pH 7.0, was performed for 10 min (UltraNeb 99; DeVilbiss, Somerset, PA, USA). In preliminary experiments, the effects of this nebulization were shown to last for >10 min but <20 min. This guided us to deliver NO at a concentration of 80 ppm for 10 min, after the nebulization of hypertonic saline. During NO inhalation, 3 mg·mL^{-1} meth was nebulized in one experiment (n=7) and 1 mg·mL^{-1} meth was nebulized in another experiment (n=6). In a third experiment, the NO concentration was increased to 300 ppm, with nebulization of 1 mg·mL^{-1} of meth (n=6). In the control experiments without NO, but with the same rabbits, there was a resting period of 10 min instead of NO inhalation before meth challenge. The meth was dissolved in sterile water and adjusted to a pH of 7.2±0.1.

Protocol 3: effects of nitric oxide inhalation on bronchoconstriction after hyperventilation with dry gas. The rabbits (n=7) were hyperventilated for 10 min with dry gas containing NO (30% O_2, 5% CO_2, 80 ppm NO in N_2). The response of the airway wall to dry air is rapid and transient over a period of 10 min [10]. The NO was therefore given during the hyperventilation. The ventilatory frequency was adjusted in order to increase minute ventilation to four times the resting condition. Ventilation was returned to the resting condition and bronchoconstriction was induced with meth at a concentration of 1 mg·mL^{-1} during NO inhalation. The rabbits served as their own controls and were challenged on another occasion with hyperventilation and meth without NO inhalation.

Statistical analysis

Statistical analysis was carried out using SigmaStat software (Jandel Scientific, Erkrath, Germany). Student's two-tailed test was used for paired data and differences within the groups were tested with one-way analysis of variance (ANOVA) for repeated measurements and Student–Newman–Keul's test. Results are presented as mean values±SEM. A value of p<0.05 was considered statistically significant.

Results

Baseline data of body weight and temperature as well as cardiac frequency (fC), MAP, $Pa,O_2/FI,O_2$, PET,CO_2, Rrs and Crs showed no significant differences between the experiments within or between protocols. To demonstrate the stability of this rabbit model, the baseline data from the first and second experiments, for all rabbits, are given in table 1.

Table 1. – Baseline data from two experiments at least 2 weeks apart for the rabbits participating in different protocols

	First experiment	Second experiment
Body weight kg	3.3±0.1	3.3±0.1
Temperature °C	38.1±0.2	38.0±0.1
fC beats·min^{-1}	221±5	220±7
MAP mmHg	70±2	69±2
Pa,O$_2$/FI,O$_2$ mmHg	79±1	80±2
PET,CO$_2$ kPa	5.4±0.1	5.3±0.1
Rrs cmH$_2$O·L^{-1}·s	50±2	52±2
Crs mL·cmH$_2$O^{-1}	4.3±0.1	4.5±0.1

Data are mean±SEM (n=16). fC: cardiac frequency; MAP: mean arterial pressure; Pa,O$_2$: arterial oxygen tension; FI,O$_2$: inspiratory oxygen fraction; PET,CO$_2$: end-tidal carbon dioxide tension; Rrs: respiratory resistance; Crs: lung compliance. (1 mmHg = 0.133 kPa.)

Protocol 1

NO at a concentration of 80 ppm reduced the bronchoconstriction in response to 3 mg·mL^{-1} of meth (p<0.05) (fig. 1a). There was no significant difference in the decrease in Crs due to meth with and without NO (3.5±0.3 mL·cmH$_2$O^{-1} with NO and 3.4±0.4 without NO). Pa,O$_2$/FI,O$_2$ decreased (p<0.05) by meth but there was no difference between the results with (68±4 kPa) and without NO (76±4 kPa). The MetHb concentration increased during NO inhalation, from 0.4±0.1 to 0.8±0.1% (p<0.01).

Protocol 2

The hypertonic saline nebulization *per se* did not affect the Rrs (52±1 before and 52±2 cmH$_2$O·L^{-1}·s after the hypertonic saline) in the rabbits studied. Meth, at a concentration of 3 mg·mL^{-1}, caused an increase in Rrs of 86±15 cmH$_2$O·L^{-1}·s after hypertonic saline nebulization (p<0.001). NO inhalation at a concentration of 80 ppm did not counteract the increase in Rrs (92±14 cmH$_2$O·L^{-1}·s) (fig. 1b).

The Rrs response to meth after nebulization of hypertonic saline was stronger than without hypertonic saline. The Rrs increase of 56±16 cmH$_2$O·L^{-1}·s from baseline (p<0.05), following 1 mg·mL^{-1} meth, was not significantly altered by inhalation of 80 ppm NO (42±11 cmH$_2$O·L^{-1}·s) (fig. 1c). Not even inhalation with NO at a concentration of 300 ppm altered the increase in Rrs from baseline in response to meth at a concentration of 1 mg·mL^{-1}. The increase in Rrs was 48±8 cmH$_2$O·L^{-1}·s (p<0.05) with NO and 44±14 cmH$_2$O·L^{-1}·s (p<0.05) without NO inhalation (fig. 1d).

Crs was significantly decreased after the hypertonic saline nebulization, from 4.5±0.1 to 3.7±0.1 mL·cmH$_2$O^{-1} (p<0.001). Meth provocation significantly decreased Crs at both 1 and 3 mg·mL^{-1} meth. Inhalation of 80 or 300 ppm NO did not significantly alter the reduction in Crs (table 2). Pa,O$_2$/FI,O$_2$ was not affected by hypertonic saline or NO inhalation but was decreased by meth provocation (p<0.001) (table 2). There was a significant increase in MetHb concentration during NO inhalation (p<0.001) (table 2). The MAP, shown in table 1, was not altered by nebulization of hypertonic saline, meth or any concentration of NO.

Protocol 3

There was a significant increase in Rrs due to meth 1 mg·mL^{-1} (p<0.05). The increase in resistance was 27±11 cmH$_2$O·L^{-1}·s^{-1} with 80 ppm NO and 28±5 cmH$_2$O·L^{-1}·s without NO (table 3). Meth at a concentration of 1 mg·mL caused a decrease in Crs (p<0.05), which was not observed during NO inhalation (table 3). Pa,O$_2$/FI,O$_2$ was increased by hyperventilation with and without NO inhalation, both to 82±2 kPa. The meth nebulization decreased the Pa,O$_2$/FI,O$_2$ ratio more with NO than without NO (p<0.05) (table 3). The MetHb concentration increased during 10 min of hyperventilation with 80 ppm NO (p<0.001) (table 3).
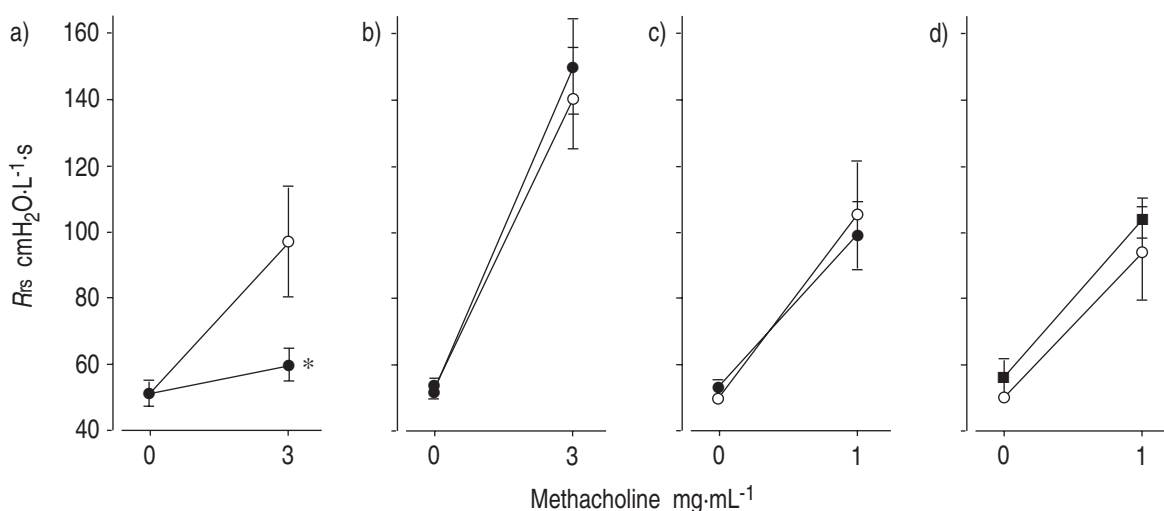

Fig. 1. – Respiratory resistance (Rrs) before and after nebulized methacholine (meth) without (○) and with nitric oxide inhalation at concentrations of 80 (●) and 300 (■) parts per million (ppm). a) Changes in Rrs induced by 3 mg·mL^{-1} meth. NO inhalation countered the increase in Rrs (*: p<0.05). b) After nebulization of hypertonic (3.6% saline) there was an increased response to meth which 80 ppm NO did not alter. c) Changes in Rrs induced by a lower concentration, 1 mg·mL^{-1}, of meth after nebulization of hypertonic saline. The increase in Rrs was the same with and without 80 ppm NO. d) A similar result was obtained with 300 ppm NO.

Table 2. – Effect of nitric oxide (NO) inhalation after nebulization of hypertonic (3.6%) saline on methacholine (meth)-induced bronchoconstriction

	3 mg·mL^{-1} Meth	3 mg·mL^{-1} Meth plus 80 ppm NO	1 mg·mL^{-1} Meth	1 mg·mL^{-1} Meth plus 80 ppm NO	1 mg·mL^{-1} plus 300 ppm NO
n	7	7	6	6	6
R_{rs} cmH$_2$O·L^{-1}·s	140±15	150±14	105±16	96±0.10	104±6
C_{rs} mL·cmH$_2$O^{-1}	1.8±0.1	1.7±0.1	3.0±0.2	2.8±0.3	3.4±0.2
$P_{a,O_2}/F_{I,O_2}$ kPa	35±4	35±2	56±5	50±5	60±9
MetHb %	0.6±0.1	0.9±0.1*	0.6±0.2	1.0±0.1*	1.9±0.2*

Data are means (±SEM). For baseline measurements see table 1. R_{rs}: respiratory resistance; C_{rs}: lung compliance; P_{a,O_2}: arterial oxygen tension; F_{I,O_2}: inspiratory oxygen fraction; MetHb: methaemoglobin; ppm: parts per million. *: p<0.05, significantly different from control situation without NO inhalation.

Discussion

The major finding in this study is that the inhibitory effect of NO inhalation on induced bronchoconstriction can be blocked, either by nebulization of hypertonic saline or by hyperventilation with dry gas. This could possibly be due to the formation of oedema, acting as a diffusion barrier or as a metabolic sink, or by the hyperosmolar airway *per se*.

One possible explanation for the inhibition of the NO effect is that the oedematous airway wall acts as a diffusion barrier for NO. Nebulization of hypertonic saline is known to increase production of mucus. Mucus on the surface of the epithelial layer may also contribute to a diffusion barrier. However, NO is extremely lipophilic [14] and its diffusing capacity in the lung is four times that of carbon monoxide [15]. It therefore seems unlikely that the transport of NO to the airway smooth muscle should have been totally blocked. Support for this argument is the significant increase in MetHb seen in the present study. If the increase in MetHb due to 300 ppm NO inhalation is compared to a previous study [6], the increases are identical with and without hypertonic saline nebulization, *i.e.* 112±4 and 112±12%, respectively. Hence, the lung vasculature and, therefore, probably also the bronchial airway wall were exposed to NO.

The mucus can act not only as a physical barrier but also as a metabolic sink for NO. Oxygen free radicals (O$_2^-$) are known to be present in the mucus [16]. NO will rapidly form the peroxynitrate anion (OONO$^-$) in contact with O$_2^-$. The O$_2^-$-rich environment of the lung, together with the low pH of the airway lining fluid, favour OONO$^-$ formation in the respiratory system [17].

Recent observations suggest that inhaled NO also reacts with thiol constituents in the surface liquid layer of the airway wall, forming stable bioactive S-nitrosothiols [17]. Therefore, reactions between NO and other cellular and chemical constituents of the airway are likely. Deactivation of the NO molecule could explain the lack of effect on airway smooth muscle found in this study.

Another possible explanation for the lack of effect of the inhaled NO could be the hyperosmolarity induced by hypertonic saline nebulization or by hyperventilation. One hypothesis is that the hyperosmolarity *per se* may inactivate the NO molecule. Further support for this comes from previous rabbit studies, where inhalation of NO at a concentration of 300 ppm decreased the MAP and the lung compliance [6, 18]. In this study, after hypertonic saline nebulization, these effects were abolished.

The increase in reactivity to meth after nebulization of hypertonic saline and hyperventilation can only be speculated. Normally, endogenous NO is involved in the inhibition bronchoconstriction. If the production of NO is blocked by NO synthase inhibitors, one could expect a hyperreactive response to the bronchial challenge. Indeed, NIJKAMP *et al.* [19] demonstrated that the *in vitro* luminal perfusion of guinea-pig tracheal tubes with NO synthesis inhibitors increased the maximum on the histamine concentration–response curve by 335%. This effect could also be achieved by removal of the airway epithelium, which is a production site for NO. Production or NO or a NO-like factor has been shown to be impaired in the rat tail artery by superfusion with hyperosmolar fluid [20]. This superfusion also gave rise to an enhancement of phenylephrine-induced contraction. Therefore, the inhibition of endogenous NO as well as exogenous NO could be one reason for the increased reactivity to induced bronchoconstriction seen in the rabbits in the present study.

In conclusion, the relaxant effect of nitric oxide inhalation on airway smooth muscle can be blocked by altering the osmolarity of the airway surface. The mechanism of this inhibition remains to be established.

Table 3. – Effect of hyperventilation with dry gas and nitric oxide (NO) inhalation on methacholine (meth)-induced bronchoconstriction

	1 mg·mL^{-1} Meth	1 mg·mL^{-1} Meth plus 80 ppm NO
n	7	7
R_{rs} cmH$_2$O·L^{-1}·s	74±6	81±10
C_{rs} mL·cmH$_2$O^{-1}	3.0±0.3	3.6±0.3
$P_{a,O_2}/F_{I,O_2}$ kPa	66±4	59±5*
MetHb %	0.5±0.1	1.5±0.1*

Data are means (±SEM). For baseline measurements see table 1. R_{rs}: respiratory resistance; C_{rs}: lung compliance; P_{a,O_2}: arterial oxygen tension; F_{I,O_2}: inspiratory oxygen fraction; MetHb: methaemoglobin; ppm: parts per million. *: p<0.05, significantly different from the control situation without NO.

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