The Effect of N^G-Nitro-L-Arginine Methyl Ester, a Nitric Oxide Synthase Inhibitor, on Respiratory Mechanics in Rats

Alessandro Rubini

Division of Physiology, Department of Anatomy and Physiology, University of Padova, Padova, Italy

Key Words

L-NAME • NO synthase • Respiratory mechanics • Work of breathing

Abstract

Background: Data describing the inhibitory effects of nitric oxide synthase (NOS) on respiratory mechanics are conflicting, and no data are available concerning possible effects on the viscoelasticity of the respiratory system, on the inspiratory work of breathing (WOB) or on respiratory system hysteresis. **Objectives:** The aim of this study was to measure the effects of NOS inhibition by N^G-nitro-L-arginine methyl ester (L-NAME) on respiratory mechanics in normal anesthetized rats. **Methods:** Using the end-inflation occlusion method, it was possible to quantify the ohmic and viscoelastic airway resistance and elastance of the respiratory system. Ohmic resistance is the normalized-to-flow pressure dissipation due to viscous forces opposing the airflow in the airways, as predicted by the Poiseuille law. Viscoelastic resistance is the normalized-to-flow pressure dissipation due to the resistance of respiratory system tissue to deformation during inflation, which is recovered after the arrest of the inspiratory flow (stress relaxation). The inspiratory WOB, its elastic and resistive components, and hysteresis were also calculated. **Results:** L-NAME induced an increment in the ohmic airway resistance and in the resistive ohmic inspiratory WOB. The viscoelastic resistance due to stress relaxation and the elastic

properties of the respiratory system were not modified, and no effect was detected on the related components of the inspiratory WOB and on hysteresis. **Conclusions:** NO acts in normal rats to reduce the ohmic component of airway resistance, decreasing the ohmic inspiratory WOB. The elastic and viscoelastic components are unaltered. Hysteresis is also unaltered, suggesting that NO has negligible effects on alveolar surfactant activity.

Copyright © 2011 S. Karger AG, Basel

Introduction

The ability of nitric oxide (NO) to relax smooth muscle cells (SMC) has been described in multiple models and muscle types, including airway smooth muscles [1].

A comprehensive review of the effects of NO as an airway SMC relaxant and of its effects against their contraction has been published previously [2]. Evidence accumulates that the relaxant effects of NO on the airways may also be linked to its activity as a neurotransmitter of the relaxant non-adrenergic, non-cholinergic system [2, 3].

Some investigators have, however, failed to demonstrate that NO inhalation reduces airway resistance in a porcine model of septic shock [4], in mechanically ventilated healthy piglets [5] and in rabbits with oleic acid-induced respiratory distress syndrome [6].

Moreover, the potent NO synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) has been found, as expected, to induce an increase in airway resistance in guinea pigs [7, 8] and pigs [9, 10], but this finding was not confirmed in rats [11] or in humans [12].

No report has been published concerning the possible effects of L-NAME on lung stress relaxation. It was hypothesized that NO causes a modulation of the activity of contractile peripheral elements in the lungs [10]. Thus, endogenous NO could have an effect on the viscoelastic properties of lung parenchyma. Stress relaxation is a complex phenomenon, exhibited by most tissues, including the lungs whose molecular basis are essentially unknown. Due to their viscoelastic properties, the tissues do not maintain constant stress under constant deformation but slowly relax. The viscoelastic pressure component of the inspiratory work of breathing (WOB) contributes significantly to the overall pressure dissipation during spontaneous breathing [13, 14]. The end-inflation occlusion method, widely used to measure respiratory mechanics [13–16], can be used to determine stress relaxation parameters. Studying factors affecting stress relaxation could also have practical connotations, since the effect of NO on reducing the viscous component of the inspiratory WOB may suggest therapeutic implications.

The hysteresis area has been found to increase in hyperoxic mechanically ventilated newborn piglets following NO inhalation [17]. L-NAME administration may thus affect the static inspiratory-expiratory pressure-volume loops, which quantify lung hysteresis, but few experiments have been carried out to verify this hypothesis.

Alterations in the inspiratory work of breathing and of its elastic and resistive components might also be another consequence of the effects of L-NAME on respiratory mechanics.

Thus, in the present experiments, L-NAME was administered to healthy rats and the end-inflation occlusion method was used to study the effects of NOS inhibition on:

- ohmic airway resistance, to confirm previous data indicating that NO has a bronchodilator effect under basal condition;
- stress relaxation of the respiratory system, in order to gain more information about poorly understood viscoelastic mechanical properties and to determine if NO can be used to reduce the viscous component of the inspiratory WOB;
- respiratory system hysteresis, possibly related to NO effects on surfactant activity and/or on other mechanisms upon which hysteresis depends, and

- inspiratory WOB, as a consequence of possible modifications in mechanical properties of the respiratory system.

Materials and Methods

The experiments were carried out on 8 Wistar albino rats of both sexes (mean weight 363 ± 32 g; 4 males and 4 females). Animals were housed and treated in accordance with the Italian law on animal experimentation (L. 116/92) and with the European Council provision 86/609/EEC. The experimental protocol was authorized by the Local Ethical Committee for Animal Experimentation (C.E.A.S.A.). Rats were anesthetized with 50 mg/100 g i.p. chloralose and laid on a heated operating table. After a tracheostomy, a small polyethylene cannula (2 mm internal diameter and 5 cm long) was inserted through an incision in the second tracheal ring and firmly secured in place. Positive pressure ventilation with room air (10 ml/kg tidal volume, V_T , 60/min breathing frequency, positive end-expiratory pressure, PEEP, 3 cm H₂O; rodent ventilator 7025; Basile, Italy) was begun, and constantly maintained throughout the experiment. The level of anesthesia was tested by checking the suppression of the corneal reflex. Limb ECG probes were placed and the rats were paralyzed (*cis*-atracurium 1 mg/100 g i.p.). Additional doses of *cis*-atracurium, a drug that does not appear to substantially alter heart rate [18], were administered as needed whenever spontaneous breathing appeared.

Positive pressure ventilation was maintained for 10 min and respiratory mechanics were then measured using the end-inflation occlusion method [13–16]. The ventilator was disconnected, positive pressure ventilation was discontinued, and the tracheal cannula was connected to a constant flow pump (SP 2000 series syringe pump sp210iw; World Precision Instruments, USA) set to deliver a V_T of 3 ml with a square wave flow (F) of 4 ml/s. The time for the rise and the fall of the flow was approximately 30 ms. The pump setting was carefully checked by directly taking measurements before beginning the experiments.

The lateral tracheal pressure proximal to the tracheal cannula was monitored (142 pc 01d; Honeywell, USA) and continuously recorded (1326 Econo Recorder; BioRad, Italy). Because abrupt changes in diameters were not present in the circuit, errors in flow resistance measurements, such as those reported previously [19], were avoided. The frequency response of the transducer and the pressure measuring system was tested by sinusoidal forcing and found to be flat up to 20 Hz. In accordance with the literature [15, 20], this frequency response was adequate to avoid mechanical artifacts in the pressure signal records.

The end-inflation occlusion method was utilized to determine the parameters of respiratory mechanics: the static elastic pressure of the respiratory system (P_{elRS}), the total resistive pressure drop (P_{maxRS}) and the sudden Newtonian resistive pressure drop at flow interruption (P_{minRS}) were measured on adequately magnified tracings (fig. 1). P_{maxRS} was calculated as the difference between the maximum value of pressure at end inflation (P_{DYNmax}) and P_{elRS} . P_{minRS} was calculated as the difference between P_{DYNmax} and P_1 , the pressure value immediately after flow interruption (fig. 1).

Fig. 1. Representative tracings of lateral tracheal pressure at flow interruption.

To avoid a viscoelastic pressure component in $P_{\min RS}$, P_1 values were identified by extrapolating the pressure tracings to the time the flow stopped. Thus, $P_{\min RS}$ represents the nearly instantaneous, Newtonian resistive pressure drop that theoretically occurs at infinite breathing frequency [21]. $P_{\min RS}$ does not include the viscoelastic pressure drop that results from stress relaxation which is, instead, included in the $P_{\max RS}$ value.

The mean pressure data obtained from the 3–5 inflations for each rat were used to calculate the respiratory system static elastance ($E_{\text{stRS}} = P_{\text{elRS}}/V_T$) and the total resistance of the respiratory system ($R_{\text{maxRS}} = P_{\text{maxRS}}/F$). The latter includes the ohmic, Newtonian inspiratory resistance to airflow offered by the airways and the resistance caused to the movement of respiratory system tissues ($R_{\text{minRS}} = P_{\text{minRS}}/F$), and the pressure drop resulting from the effect of viscoelastic resistance and stress relaxation. This last component of R_{maxRS} was isolated and quantified as ‘viscous’ resistance ($R_{\text{viscRS}} = R_{\text{maxRS}} - R_{\text{minRS}}$). Ohmic resistance is the normalized-to-flow pressure dissipation due to viscous forces opposing the airflow in the airways, as predicted by the Poiseuille law. Viscoelastic resistance is the normalized-to-flow pressure dissipation due to the resistance of respiratory system tissue to deformation during inflation, which is recovered after the arrest of the inspiratory flow (stress relaxation).

The equipment resistance, including the tracheal cannula and the standard three-way stopcock, was measured separately at a flow rate of 4 ml/s and amounted to 0.0575 cm H₂O/(ml/s) (R_{eq}). All inflations were performed at a fixed flow rate of 4 ml/s, and R_{eq} was subtracted from the results (intrinsic values).

The work of breathing (WOB_R) values were calculated as previously described [16, 22–24] and outlined in figure 2. Static pressure-volume relationships were assumed to be linear up to an inflation volume of 3 ml. This measurement is in agreement with published data showing essentially linear pressure-volume relationships for the rat lung within this volume range [16, 24, 25]. The validity of this assumption has also been confirmed experimentally by the essentially linear pressure-volume relationships

Fig. 2. Schematic drawing of the pressure-volume relationship during constant flow inflation and flow interruption. Area 1 = WOB_{elRS} ; areas 2 + 3 = WOB_{resRS} (= WOB_{elRS} + WOB_{resRS}); area 3 = WOB_{ohmRS} , dissipated to overcome ohmic airway resistance plus the viscous resistance to the movement opposed by the lungs and chest wall tissues. $WOB_{resRS} - WOB_{ohmRS} = WOB_{viscRS}$, the mechanical work done to overcome the resistive effects of stress relaxation.

observed during static inflations performed for HyRS measurements up to a total inflation volume of 5 ml (see below). The total WOB ($\text{WOB}_{\text{totRS}}$) was calculated by measuring the surface areas delimited by the total pressure (PDYN) tracings from which the resistive pressure due to the tracheal cannula and the three-way stopcock was subtracted. The elastic WOB (WOB_{elRS}) was obtained on the same diagram as the areas encompassing the static pressure-volume lines. The WOB ($\text{WOB}_{\text{resRS}}$) was obtained by subtraction: $\text{WOB}_{\text{resRS}} = \text{WOB}_{\text{totRS}} - \text{WOB}_{\text{elRS}}$. $\text{WOB}_{\text{resRS}}$ was also partitioned into the resistive work required to overcome ohmic airway resistance and respiratory system tissue movements ($\text{WOB}_{\text{ohmRS}}$) and the resistive work required to overcome the effects of stress relaxation ($\text{WOB}_{\text{viscRS}}$). $\text{WOB}_{\text{ohmRS}}$ was calculated as the area comprised between the PDYN and P_1 lines, where P_1 is the pressure value immediately after flow interruption (fig. 1). $\text{WOB}_{\text{viscRS}}$ was calculated by subtraction as $\text{WOB}_{\text{viscRS}} = \text{WOB}_{\text{resRS}} - \text{WOB}_{\text{ohmRS}}$. Calculations were made for each rat and the mean values were statistically compared.

After respiratory mechanics were measured, which took approximately 1 min, mechanical ventilation was restored and maintained for 5 min. To obtain a constant volume history for the measurement of respiratory system hysteresis, the respiratory system

Fig. 3. Temporal sequence of the experimental procedure.

was inflated with a 10-ml syringe to a static elastic pressure of 20–25 cm H₂O three times. After that, the respiratory system was inflated, and subsequently deflated, five times in 1-ml steps using a precision glass syringe starting from the functional residual capacity (FRC). Each volume was maintained at each step for approximately 5–6 s. The relevant static elastic pressures were measured, and the static inflation-deflation volume-pressure curves were determined. The hysteresis areas (HyRS) were quantified using electronic digital integration (expressed as cm H₂O·ml).

The ventilator was disconnected and PEEP was neither present during the constant flow inflations to measure respiratory mechanical parameters nor during the 1-ml inflations and deflations to measure HyRS.

The measurements and calculations described above were repeated for each rat 10 and 20 min following 50 mg/kg L-NAME intraperitoneal administration, dissolved in 1 ml saline.

Even though data in the literature indicate that the mechanical ventilation parameters here adopted are not injurious to the respiratory system [23], the experiments were also carried out in 6 control rats, considered a sufficient number for this purpose, to exclude the possibility that any observed alterations in the study group was due to a time-related effect. The measurements and calculations were thus repeated before, and 10 and 20 min after intraperitoneal administration of 1 ml saline in the control rats, not significantly different from the experimental rats with regard to mean weight and sex distribution.

The temporal sequence of the experimental procedure for experimental and control groups is summarized in figure 3.

All the data regarding the measurements and calculations made before and after L-NAME or saline administration were normally distributed (Smirnov-Kolmogorov test). The a priori hypothesis was that the data collected before and after L-NAME or saline administration were identical, and the statistical analysis rejected it for L-NAME-treated rats (see below). Statistical analysis was performed using multivariate ANOVA and post hoc (Newman-Keuls) tests, and the data are reported as mean values $\pm$ SE.

Results

Significant increments in $R_{\min RS}$ and in the related $WOB_{ohm RS}$ mean values were observed 10 and 20 min following intraperitoneal injections in the L-NAME-treated rats (tables 1, 2). $WOB_{ohm RS}$ values are directly related to

$R_{\min RS}$ values because they represent the mechanical work done to overcome the viscous forces opposing the movement of the inspiratory flow in the airway. This is also shown by the schematic drawing depicted in figure 2, showing that $WOB_{ohm RS}$ was calculated on the basis of the observed $P_{\min RS}$ values, which directly determine $R_{\min RS}$.

Other respiratory mechanical parameters were not modified significantly over time (tables 1, 2), and the mean heart rate values were also unaltered.

The statistical analysis of the data obtained from control rats showed no significant difference between the mean values of the measured and calculated respiratory mechanical parameters before and after saline administration. Heart rate mean values also did not significantly differ over time.

Discussion

The experiments outlined here indicate that there is a significant increment in $R_{\min RS}$ following NOS inhibition by L-NAME. In agreement with previously described findings in guinea pigs [7, 8] and in newborn piglets [10], these data confirm that NO has a relaxing effect on airway smooth muscle in normal rats in basal conditions.

The present results are in contrast with some data previously reported, but in the experiments by Dahm et al. [4] and Zhu et al. [6], the absence of effects of NO inhalation on respiratory system resistance was evaluated in endotoxin- or oleic acid-induced lung injury, respectively, and Zhang et al. [5] simply reported that NO inhalation did not adversely affect basal respiratory system resistance. In the experiments by Kips et al. [11] and Maniscalco et al. [12], no significant effect of L-NAME was detected on basal respiratory system resistance in healthy rats or humans, respectively, but the L-NAME dosages were much lower than the dosage presently used.

Table 1. Mean values ($\pm$ SE, n = 8) of respiratory mechanics in experimental rats

Respiratory parameters	Time					
	-5 min	0	10 min	15 min	20 min	25 min
E_{stRS} , cm H ₂ O/ml	2.9 $\pm$ 0.2		2.9 $\pm$ 0.2		3 $\pm$ 0.2	
R_{maxRS} , cm H ₂ O/ml $\cdot$ s ⁻¹	1.1 $\pm$ 0.1		1.1 $\pm$ 0.1		1.2 $\pm$ 0.1	
R_{minRS} , cm H ₂ O/ml $\cdot$ s ⁻¹	0.26 $\pm$ 0.04		0.33 $\pm$ 0.04*		0.44 $\pm$ 0.05**	
R_{viscRS} , cm H ₂ O/ml $\cdot$ s ⁻¹	0.85 $\pm$ 0.1		0.8 $\pm$ 0.1		0.8 $\pm$ 0.1	
HyRS, cm H ₂ O $\cdot$ ml		4.5 $\pm$ 0.3		4.3 $\pm$ 0.5		4.7 $\pm$ 0.4
Heart rate, beats/min	302 $\pm$ 22		337 $\pm$ 18		320 $\pm$ 22	

Data are shown for different times following 50 mg/kg i.p. L-NAME administration (time = 0). The first HyRS measurement was obtained immediately before intraperitoneal injection. * $p < 0.05$ and ** $p < 0.01$, vs. before the intraperitoneal injection (ANOVA). Differences between the mean values observed at 10 and 20 min were not significant.

Some reports have been published describing no significant effects of inhaled NO on respiratory system resistance in preterm infants [26, 27], also affected by the respiratory syncytial virus [28]. Belik et al. [29] showed that the content and the activity of the NOS competitor arginase in the rat lungs exhibit significant reduction with increasing age of the animals, from fetal life to newborns and adults. Thus, significant effects of NO on respiratory mechanics are more likely to be expected in the presently used adult animals than in the newborns or infants.

The mechanisms underlying the action of NO include [2]:

- a cGMP-dependent mechanism inhibiting Ca²⁺ release from the sarcoplasmic reticulum of airway SMC and inducing a reduction in the sensitivity of the contractile system to Ca²⁺ intracellular concentration;
- a cGMP-independent mechanism of direct activation of K_{Ca} channels leading to SMC hyperpolarization and relaxation.

Recently, it was shown that salbutamol-induced relaxation in rat tracheas involves the relaxing action of NO [30].

WOB_{ohmRS} is significantly higher following R_{minRS} increase (table 2). These findings suggest that in basal conditions NO acts by reducing the ohmic resistive component of inspiratory WOB during spontaneous breathing too, thus reducing the inspiratory muscle mechanical load. The effect is, however, limited to the ohmic component of WOB as the viscous one is not significantly modified following NOS inhibition (table 2).

According to a previous report [10], NO is associated with the modulation of the activity of contractile periph-

Table 2. Mean values ($\pm$ SE, n = 8) of WOB and its elastic and resistive components

WOB	Time		
	-5 min	10 min	20 min
WOB _{totRS} , cm H ₂ O $\cdot$ ml	26.7 $\pm$ 2.3	27.2 $\pm$ 1.8	27.8 $\pm$ 2.4
WOB _{elRS} , cm H ₂ O $\cdot$ ml	12.9 $\pm$ 1.1	12.9 $\pm$ 1	13.7 $\pm$ 1
WOB _{resRS} , cm H ₂ O $\cdot$ ml	13.8 $\pm$ 1.5	14.2 $\pm$ 1.2	14.1 $\pm$ 1.8
WOB _{ohmRS} , cm H ₂ O $\cdot$ ml	4.6 $\pm$ 0.4	5.5 $\pm$ 0.4*	6.5 $\pm$ 0.6*
WOB _{viscRS} , cm H ₂ O $\cdot$ ml	9.1 $\pm$ 1.1	8.8 $\pm$ 0.9	7.6 $\pm$ 1.4

Data are shown at different times from 50 ml/kg i.p. L-NAME injection (time 0). * $p < 0.05$ vs. before injection (ANOVA). Differences between the mean values observed 10 and 20 min following the injection were not significant.

WOB_{ohmRS} values are directly related to R_{minRS} values because they represent the mechanical work done to overcome viscous forces opposing the movement of the inspiratory flow in the airways. In the same way, WOB_{elRS} values are directly related to E_{stRS} values because they represent the elastic mechanical work done to overcome the elastic forces opposing respiratory system inflation.

eral elements in the lungs, suggesting that it may play a role in determining the viscous properties of lung parenchyma, but the data presented here do not confirm this hypothesis.

In basal conditions, the viscous component represented by stress relaxation substantially contributes to WOB_{totRS} [13, 14, 23, 24], but its molecular mechanism(s) remain largely unclear and poorly understood.

It has been shown that it is increased as an effect of increased lung blood volume and flow [16] in inflamed

lung tissue [23, 31–33] and as an effect of body warming [24], but it is not influenced by the estrous cycle in the rat [34]. The present findings indicating that stress relaxation phenomena are independent of NO activity have further increased the knowledge about the viscoelastic properties of the respiratory system. They also show that L-NAME administration does not modify hysteresis surface area of volume-pressure loops (table 1).

Hysteresis is a complex phenomenon depending upon the plasticity of the tissue elements, surface hysteresis linked to alveolar surfactant activity, and differences in the sequential recruitment and derecruitment of lung units between inflation and deflation.

Although different mechanisms may affect hysteresis, the present data suggest that alveolar surfactant activity is independent of NO, as previously shown by Zhang et al. [5] in mechanically ventilated healthy piglets.

The same result has been also confirmed by data obtained by Zhu et al. [6] on a rabbit experimental model of oleic acid-induced respiratory distress syndrome: no effect of isolated NO inhalation on the bronchoalveolar lavage fluid content of tensioactive desaturated phosphatidylcholine was detected. In contrast, Storme et al. [17] reported increased hysteresis areas as an effect of NO inhalation, but their data were probably influenced by the confounding effects of long-lasting 100% oxygen inhalation.

E_{stRS} was likewise not significantly modified (table 1), suggesting that NO does not significantly affect the overall elastic properties of the respiratory system. This result confirms the data reported by Storme et al. [17] in hyperoxic mechanically ventilated newborn piglets. Beyond the alveolar wall-alveolar gas surface forces, the elastic reaction to distension of the respiratory system depends on the elastin content of the alveolar wall and on its stress-strain relationship. Data were reported indicating that NO has no effect on the elastin content or on the ensuing elastic behavior of the arterial wall [35, 36], and present results suggest that the same may also be true for the alveolar wall.

The constancy of the stress relaxation-linked viscous component of resistance, and of the elastance of the respiratory system, is reflected by the nonsignificant alterations observed in WOB_{viscRS} and WOB_{elRS} mean values following L-NAME administration (table 2).

The end-inflation occlusion method has been widely used to study respiratory mechanics in experimental animals [16, 20, 23, 24, 34, 37] and in humans [13–15, 22]. Modelling the respiratory system as a two-compartment model makes it possible to measure the static elastance

and the total resistive pressure dissipation. The total resistive pressure dissipation includes the effects of the ohmic resistance of the airways and the effects of additional resistance resulting from stress relaxation, which can be calculated by subtraction.

The absolute values of the parameters of respiratory mechanics listed here are similar to those previously measured during analogous experiments [16, 20, 23, 24, 34, 37].

Ideally, the inflation flow should stop instantaneously, but this is practically impossible to achieve. By means of a procedure proposed by Bates et al. [21] and utilized here, pressure tracings are manually extrapolated to account for the time needed to completely halt the inspiratory flow, and as a result the possibility of errors is almost negligible [16, 20, 23, 24].

P_{minRS} represents the nearly instantaneous, Newtonian resistive pressure drop which theoretically occurs at infinite breathing frequency, i.e. without the viscoelastic, time-related pressure drop due to stress relaxation, which is instead included in the P_{maxRS} value. Researchers have thus considered P_{minRS} to be the pressure driving the airway flow immediately before its interruption [13, 14, 23, 24].

The procedure adopted to calculate $WOBRS$ and its components introduced an approximation due to the well-known volume dependence of resistive pressures in the respiratory system, the effects of which were not considered here. However, on the basis of data published previously [15], this approximation should have only minor effects, also due to the small volume change in the current study. Moreover, these effects would be expected to affect the results obtained before and after L-NAME administration equally and thus would not be predicted to introduce systematic error.

Previous studies have demonstrated that the estrous cycle may influence $HyRS$ [34] but not other respiratory mechanical parameters [34]. The effects of the estrous cycle on the measurements made here were negligible, because of the short time frame in which the effects of intraperitoneal L-NAME administration were examined.

The mechanical ventilation settings used during these experiments were the same as those described as ‘non-injurious’ in the literature [23, 24]. In particular, ‘non-injurious’ ventilation lasting 1 h has been shown to cause neither alterations in respiratory system mechanics nor modifications in $HyRS$ values [23, 24]. The control measurements listed here have confirmed that the rats had no change in respiratory mechanical parameters over the time span that the experiments lasted. The present results

were not influenced, therefore, by the injurious effects that longer-term mechanical ventilation might per se exert.

Mean heart rate values outlined in table 1 suggest that the rats were in generally stable conditions during the experimental procedures. Significant alterations in the sympathetic nervous system tone, known to affect airway resistance, may in particular be excluded.

Intraperitoneal administration of L-NAME is a well-documented procedure [38–40], and the dosage utilized here was chosen based on previously published results to obtain a significant inhibition of NOS activity [38–41]. In particular, Cheng et al. [39] demonstrated significantly attenuated NO production and NOS mRNA expression in rats treated with 10 mg/kg i.p. L-NAME, a lower dosage than the one used in this study. In addition, in guinea pigs 50 mg/kg induced a significant reduction in the amount

of NO exhaled [41]. Thus, even if it is not possible to exactly define if 50 mg/kg of L-NAME lead to a complete NOS blockade, this dosage was demonstrated to significantly reduce NO production, which is what the present study design requested.

In conclusion, NOS inhibition increases ohmic resistance of the airways and the related WOBRS in normal rats, indicating that NO exerts a physiological role in causing bronchodilation. The present results also suggest a therapeutic use of NO and NO donors, addressing the possibility of their use to obtain a decrease in airway resistance.

Financial Disclosure and Conflict of Interest

The author has no conflict of interest to declare.

References

- Carvajal JA, Germain AM, Huidobro-Toro JP, Weiner CP: Molecular mechanism of cGMP-mediated smooth muscle relaxation. *J Cell Physiol* 2000;184:409–420.
- Ricciardolo FLM, Sterk PJ, Gaston B, Folkerts G: Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;84:731–765.
- Barnes PJ, Belvisi MG: Nitric oxide and lung disease. *Thorax* 1993;48:1034–1043.
- Dahm PL, Jonson B, De Robertis E, Myhre E, Svantesson C, Thorne J, Blomquist S: The effects of nitric oxide inhalation on respiratory mechanics and gas exchange during endotoxaemia in the pig. *Acta Anaesthesiol Scand* 1998;42:536–544.
- Zhang H, Lindwall R, Zhu L, Frostell C, Sun B: Lung physiology and histopathology during cumulated exposure to nitric oxide in combination with assisted ventilation in healthy piglets. *Pulm Pharmacol Ther* 2003;16:163–169.
- Zhu GF, Sun B, Niu SF, Cai YY, Lin K, Lindwall R, Robertson B: Combined surfactant therapy and inhaled nitric oxide in rabbits with oleic acid-induced acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1998;158:437–443.
- Prado CM, Leick-Maldonado EA, Kasahara DI, Capelozzi VL, Martins MA, Tibério IF: Effect of acute and chronic nitric oxide inhibition in an experimental model of chronic pulmonary allergic inflammation in guinea pigs. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L677–L683.
- Prado CM, Leick-Maldonado EA, Yado L, Leme AS, Capelozzi VL, Martins MA, Tibério IF: Effect of nitric oxide synthases in chronic allergic airway inflammation and remodelling. *Am J Respir Cell Mol Biol* 2006;35:457–465.
- Khassawneh MY, Dreshaj IA, Liu S, Chang CH, Haxhiu MA, Martin RJ: Endogenous nitric oxide modulates responses of tissue and airway resistance to vagal stimulation in piglets. *J Appl Physiol* 2002;93:450–456.
- Potter CF, Dreshaj IA, Haxhiu MA, Stork EK, Chatbun RL, Martin RJ: Effect of exogenous and endogenous nitric oxide on the airway and tissue components of lung resistance in the newborn piglet. *Pediatr Res* 1997;41:886–891.
- Kips JC, Lefebvre RA, Peleman RA, Joos GF, Pauwels RA: The effect of a nitric oxide synthase inhibitor on the modulation of airway responsiveness in rats. *Am J Respir Crit Care Med* 1995;151:1165–1169.
- Maniscalco M, Sofia M, Smith A, Demoncheaux EA, Mormile A, Faraone S, Higenbottam T: Lack of effect of nitric oxide inhibition on bronchial tone and methacholine-induced bronchoconstriction in man. *Respir Med* 1997;91:335–340.
- Bates JHT, Rossi A, Milic-Emili J: Analysis of the behavior of the respiratory system with constant inspiratory flow. *J Appl Physiol* 1985;58:1840–1848.
- Bates JHT, Baconnier P, Milic-Emili J: A theoretical analysis of interrupter technique for measuring respiratory mechanics. *J Appl Physiol* 1988;64:2204–2214.
- D'Angelo E, Calderini E, Torri G, Robatto M, Bono D, Milic-Emili J: Respiratory mechanics in anesthetized paralyzed humans: effects of flow, volume, and time. *J Appl Physiol* 1989;67:2556–2564.
- Rubini A, Gasperetti A, Catena V, Del Monte D: Effects of acute blood volume expansion on respiratory mechanics in the rat. *Respiration* 2010;79:497–505.
- Storme L, Riou Y, Dubois A, Fialdès P, Jailard S, Klosowsky S, Dupuis B, Lequien P: Combined effects of inhaled nitric oxide and hyperoxia on pulmonary vascular permeability and lung mechanics. *Crit Care Med* 1999;27:1168–1174.
- Buzello W, Diefenbach C, Nigrovic W: Muscle relaxants: a clinical update. *Acta Anaesthesiol Scand Suppl* 1996;109:165–167.
- Chang HK, Mortola JP: Fluid dynamic factors in tracheal pressure measurements. *J Appl Physiol* 1981;51:218–225.
- Reta GS, Riva JA, Piriz H, Medeiros AS, Rocco PMR, Zin WA: Effects of halothane on respiratory mechanics and lung histopathology in normal rats. *Br J Anaesth* 2000;84:372–377.
- Bates JHT, Hunter IW, Sly PD, Okubo S, Filiault S, Milic-Emili J: Effect of valve closure time on the determination of respiratory resistance by flow interruption. *Med Biol Eng Comput* 1987;25:136–140.
- Coussa ML, Guerin C, Eissa NT, Corbeil C, Chassé M, Braidy J, Matar M, Milic-Emili J: Partitioning of work of breathing in mechanically ventilated COPD patients. *J Appl Physiol* 1993;75:1711–1719.

- 23 Rubini A: IL-6 increases the airway resistance in the rat. *Cytokine* 2010;51:266–273.
- 24 Rubini A: The effect of body warming on respiratory mechanics in rats. *Respir Physiol Neurobiol* 2011;175:255–260.
- 25 Diamond L, O'Donnell M: Pulmonary mechanics in normal rats. *J Appl Physiol* 1977; 43:942–948.
- 26 Di Fiore JM, Hibbs AM, Zadell AE, Merrill JD, Eichenwald EC, Puri AR, Mayock DE, Courtney SE, Ballard RA, Martin RJ: The effect of inhaled nitric oxide on pulmonary function on preterm infants. *J Perinatol* 2007;27:766–771.
- 27 Athavale K, Claure N, D'Ugard C, Everett R, Swaminathan S, Bancalari E: Acute effects of inhaled nitric oxide on pulmonary and cardiac function in preterm infants with evolving bronchopulmonary dysplasia. *J Perinatol* 2004;24:769–774.
- 28 Patel NR, Hammer J, Nichani S, Numa A, Newth CJ: Effect of inhaled nitric oxide on respiratory mechanics in ventilated infants with RSV bronchiolitis. *Intensive Care Med* 1999;25:81–87.
- 29 Belik J, Shehnaz D, Pan J, Grasemann H: Developmental changes in arginase expression and activity in the lung. *Am J Physiol Lung Cell Mol Physiol* 2008;294:L498–L504.
- 30 Montalvo F, Cantres-Fonseca O, Santos G, Vega M, Torres I, Carmona J, Dexter D, Santacana G: Nitric oxide is involved in the response of the isolated intact and epithelium-denuded rat trachea to β_2 adrenergic receptor agonist salbutamol. *Respiration* 2010; 80:426–432.
- 31 Eissa RT, Ranieri VM, Corbeil C, Chassè M, Robatto FM, Braidy J, Milic-Emili J: Analysis of behaviour of the respiratory system in ARDS patients: effects of flow, volume and time. *J Appl Physiol* 1991;70:2719–2729.
- 32 Guérin C, Coussa ML, Eissa NT, Corbeil C, Chassè M, Braidy J, Matar N, Milic-Emili J: Lung and chest wall mechanics in mechanically ventilated COPD patients. *J Appl Physiol* 1993;74:1570–1580.
- 33 Tantucci C, Corbeil C, Chassè M, Robatto FM, Nava S, Braidy J, Matar N, Milic-Emili J: Flow and volume dependence of respiratory system flow resistance in patients with adult respiratory distress syndrome. *Am Rev Respir Dis* 1992;145:355–360.
- 34 Rubini A, Bondi M: Effect of the oestral cycle on respiratory mechanics in the rat. *Acta Physiol* 2007;189:379–383.
- 35 Augier T, Bertolotti C, Friggi A, Charpiot P, Barlatier A, Bodard H, Chareyre C, Guillou J, Luccioni R, Garçon D, Rolland PH: Therapeutic effects of nitric oxide-donor isosorbide dinitrate on atherosclerosis-induced alterations on hemodynamics and arterial viscoelasticity are independent of the wall elastic component. *J Cardiovasc Pharmacol* 1996;27:752–759.
- 36 Fitch RM, Rutledge JC, Wang YX, Powers AF, Tseng JL, Clary T, Rubanyi GM: Synergistic effect of angiotensin II and nitric oxide synthase inhibitor in increasing aortic stiffness in mice. *Am J Physiol Heart Circ Physiol* 2006;290:H1190–H1198.
- 37 Peratoner A, Nascimento CS, Santana MCE, Cadete RA, Negri EM, Gullo A, Rocco PRM, Zin WA: Effects of propofol on respiratory mechanics and lung histology in normal rats. *Br J Anaesth* 2004;92:737–740.
- 38 Chen Y, Boettger MK, Reif A, Schmitt A, Uceyler N, Sommer C: Nitric oxide synthase modulates CFA-induced thermal hyperalgesia through cytokine regulation in mice. *Mol Pain* 2010;2:6–13.
- 39 Cheng S, Yan WM, Yang B, Shi JD, Song MM, Zhao Y: A crucial role of nitric oxide in acute lung injury secondary to the acute necrotizing pancreatitis. *Hum Exp Toxicol* 2010;29: 329–337.
- 40 Hosseini M, Sadeghnia HR, Salehabadi S, Alavi H, Gorji A: The effect of L-arginine and L-NAME on pentylenetetrazole induced seizures in ovariectomized rats, an in vivo study. *Seizures* 2009;18:695–698.
- 41 Pennacchioni-Alves P, Vieira RP, Santos Lopes FD, Arantes-Costa FM, Pianheri FB, Martins MA, Fernandes Carvalho CR: Role of nitric oxide in hyperpnea-induced bronchoconstriction and airway microvascular permeability in guinea pigs. *Exp Lung Res* 2010;36:67–74.