Positive End-Expiratory Pressure Ventilation Elicits Increases in Endogenously Formed Nitric Oxide as Detected in Air Exhaled by Rabbits

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Background: Nitric oxide (NO) formed from l-arginine is exhaled by mammals and regulates pulmonary vascular tone. Little is known about how its formation is stimulated.

Methods: The concentration of NO in exhaled air was monitored by chemiluminescence in pentobarbital-anesthetized rabbits receiving mechanical ventilation by tracheostomy with graded positive end-expiratory pressure (PEEP).

Results: Introduction of PEEP (2.5–15 cmH2O) elicited dose-dependent and reproducible increments in exhaled NO and in arterial oxygen tension (PaO2). The increase in exhaled NO exhibited a biphasic pattern, with an initial peak followed by a partial reversal during the 4-min period at each level of PEEP. Thus, at a PEEP of 10 cmH2O, exhaled NO initially increased from 19 ± 4 to 30 ± 5 parts per billion (ppb) (P < 0.001, n = 9) and then decreased to 27 ± 5 ppb (P < 0.005) at the end of the 4-min observation period. Simultaneously, PaO2 increased from 72 ± 12 mmHg in the control situation to 105 ± 11 mmHg (P < 0.05) at a PEEP of 10 cmH2O. After bilateral vagotomy, including bilateral transection of the depressor nerves, the increase in exhaled NO in response to PEEP was significantly reduced (P < 0.01). Thus, after vagotomy, a PEEP of 10 cmH2O elicited an increase in the concentration of exhaled NO from 11 ± 5 to 17 ± 3 ppb (n = 7). Vagotomy did not affect the baseline concentration of NO in exhaled air. The PEEP-induced increments in PaO2 were not affected by the NO synthase inhibitor l-N-arginine-methylster (30 mg; kg−1 intravenously).

Conclusions: PEEP elicited increments in exhaled NO, perhaps by a stretch-dependent effect on the respiratory system. This finding may be attributed in part to a vagally influenced mechanism. (Key words: Lung(s); stretch; Mediators; nitric oxide. Nerves, vagus; vagotomy. Ventilation: positive end-expiratory pressure.)

INCREASED expiratory pressure is used in patients whose lungs are mechanically ventilated (positive end-expiratory pressure [PEEP]) and in spontaneously breathing patients (continuous positive airway pressure [CPAP]) with pulmonary disease in an attempt to improve the oxygenation of blood. The beneficial effect on oxygenation by PEEP or CPAP is attributed to an increase in functional residual capacity.¹ Thus, the opening of previously closed alveoli is probably the greatest single advantage of PEEP or CPAP.² In addition, PEEP or CPAP decreases airway resistance according to the inverse relation between lung volume and airway resistance.³ Accordingly, PEEP is known to reduce shunt blood flow in the lungs.³ Furthermore, reductions in lung water by PEEP or CPAP have been proposed,⁴ although that effect remains controversial.⁵

There is evidence indicating that endogenous nitric oxide (NO) plays a key role in pulmonary function. Thus, NO has been demonstrated to participate in pulmonary vascular regulation.⁶,⁷ Furthermore, NO formed from l-arginine and likely derived from epithelium or nerves within the respiratory system can be detected in exhaled air, with stable concentrations at rest.¹¹ In humans a substantial part of the NO detected in exhaled air is derived from the upper airways.¹² Increased concentrations of exhaled NO have been found in response to exposure of the lower airways to the bronchoconstric
strictive agent prostaglandin F₂α, and during bronchoconstriction induced by allergen in sensitized animals. In addition, increased amounts of NO can be detected in exhaled air during exercise. Despite these studies little is known about how changes in exhaled NO are actually stimulated.

The aim of the current study was to investigate whether PEEP affects lung formation of NO, and if it does, to elucidate vagal influences in this process. A second objective was to study the effects of PEEP on arterial blood gases before and after inhibition of endogenous NO formation.

**Materials and Methods**

The experiments were approved by the local animal ethics committee. Fourteen New Zealand White rabbits (2–3 kg) were anesthetized with pentobarbital sodium (6 mg · ml⁻¹, 50–60 mg · kg⁻¹ body weight; Mebumal Vct., Nord Vacc, Uppsala, Sweden) via an ear vein. Breathing was facilitated by tracheal cannulation, and the animals lungs were ventilated with a constant-volume ventilator (683, Harvard, South Natick, MA) adjusted to keep blood gases normal (40 breaths · min⁻¹, 250 ml · min⁻¹, fraction of inspired oxygen 0.21). The air supplied to the ventilator was rendered free of NO (<1 part per billion [ppb]) by filtering through a large charcoal filter (150 × 12 cm). Catheters containing heparin (500 IU · ml⁻¹; Kabi Pharmacia, Stockholm, Sweden) were inserted in the left carotid artery for blood pressure recordings and in the right jugular vein for administration of a continuous infusion of glucose (2.5 g · 100 ml⁻¹), dextran 70 (3.0 g · 100 ml⁻¹), sodium bicarbonate (0.7 g · 100 ml⁻¹), and pentobarbital sodium (360 mg · 100 ml⁻¹) at 5 ml · kg⁻¹ · h⁻¹ by means of a syringe pump (STC-521, Terumo, Tokyo, Japan). Blood pressure was recorded with a pressure transducer (Statham, Hato Rey, Puerto Rico) and polygraph (Grass, Quincy, MA). Rectal temperature was maintained at 37–38°C by means of a heating pad connected to a thermostat (Wittman-Hereaus, Heidelberg, Germany). Drugs were infused through the venous catheter and administered by a microinfusion pump (CMA 100, Carnegie Medical, Stockholm, Sweden).

PEEP was achieved by positioning the end of a tube connected to the outlet of the ventilator into a precalibrated water container. A 4-min period was allowed at each PEEP level, and measurements were performed after 1 and 4 min of PEEP. Increasing levels of PEEP were applied in a cumulative fashion (fig. 1). When the effect of L-N⁴-arginine-methyl ester (L-NAME) (Sigma, St. Louis, MO) or vagotomy was studied, two dose–response curves for increment levels of PEEP were applied, with a 15-min resting period allowed between them. Thereafter, vagotomy or slow intravenous injection of L-NAME was performed, after which a third dose–response curve for the PEEP-induced increases in NO was obtained.

NO was analyzed on a chemiluminescence system (NOA 270, Sievers, Boulder, CO) set at an integration time of 0.12 s. A gas flow of 150 ml · min⁻¹, controlled by a precision flow meter (Brooks Instrument B. V., Veenendaal, Netherlands) and a working pressure of 7–9 mmHg was obtained by a vacuum pump (18 Two Stage, Edwards High Vacuum, Crawley, Sussex, United Kingdom). The NO analyzer was calibrated by means of mass flow controllers (Bronkhorst, Ruurlo, Holland), with dilutions in filtered air of a certified NO standard gas in nitrogen (AGA Specialgas, Lidingo, Sweden). The final concentration of NO in the calibration gas was 2.5, 10, 50, 100, and 200 ppb (accuracy ± 5%). Sampling of exhaled air was performed by means of a catheter positioned within the tracheostomy tube. The NO analyzer was supplied with a gas flow of 150 ml · min⁻¹ of exhaled air. In control experiments in which the animal was replaced with a rubber balloon attached to the tracheostomy tube, and ventilated with a fixed concentration of NO (30 ppb), application of PEEP did not affect the measured concentration of NO. Thus, the observed changes in NO concentration are unlikely to be the result of NO scavenging or loss.

![PEEP cmH₂O](image)

**ENDOGENOUS**

Arterial blood pressure was measured via a femoral artery catheter, and above the aortic arch by means of a fluid-filled catheter (1.8 mm, 1.6 mm, 0.9 mm, 0.6 mm, 0.3 mm, 0.2 mm, 0.1 mm, 0.07 mm, 0.05 mm, 0.03 mm, 0.02 mm, 0.01 mm, 0.007 mm, 0.005 mm, 0.003 mm, 0.002 mm, 0.001 mm, 0.0007 mm, 0.0005 mm, 0.0003 mm, 0.0002 mm, 0.0001 mm, 0.00007 mm, 0.00005 mm, 0.00003 mm, 0.00002 mm, 0.00001 mm, 0.000007 mm, 0.000005 mm, 0.000003 mm, 0.000002 mm, 0.000001 mm, 0.0000007 mm, 0.0000005 mm, 0.0000003 mm, 0.0000002 mm, 0.0000001 mm, 0.00000007 mm, 0.00000005 mm, 0.00000003 mm, 0.00000002 mm, 0.00000001 mm, 0.000000007 mm, 0.000000005 mm, 0.000000003 mm, 0.000000002 mm, 0.000000001 mm, 0.0000000007 mm, 0.0000000005 mm, 0.0000000003 mm, 0.0000000002 mm, 0.0000000001 mm, 0.00000000007 mm, 0.00000000005 mm, 0.00000000003 mm, 0.00000000002 mm, 0.00000000001 mm, 0.000000000007 mm, 0.000000000005 mm, 0.000000000003 mm, 0.000000000002 mm, 0.000000000001 mm, 0.0000000000007 mm, 0.0000000000005 mm, 0.0000000000003 mm, 0.0000000000002 mm, 0.0000000000001 mm, 0.00000000000007 mm, 0.00000000000005 mm, 0.00000000000003 mm, 0.00000000000002 mm, 0.00000000000001 mm, 0.000000000000007 mm, 0.000000000000005 mm, 0.000000000000003 mm, 0.000000000000002 mm, 0.000000000000001 mm, 0.0000000000000007 mm, 0.0000000000000005 mm, 0.0000000000000003 mm, 0.0000000000000002 mm, 0.0000000000000001 mm, 0.00000000000000007 mm, 0.00000000000000005 mm, 0.00000000000000003 mm, 0.00000000000000002 mm, 0.00000000000000001 mm, 0.000000000000000007 mm, 0.000000000000000005 mm, 0.000000000000000003 mm, 0.000000000000000002 mm, 0.000000000000000001 mm, 0.0000000000000000007 mm, 0.0000000000000000005 mm, 0.0000000000000000003 mm, 0.0000000000000000002 mm, 0.0000000000000000001 mm, 0.00000000000000000007 mm, 0.00000000000000000005 mm, 0.00000000000000000003 mm, 0.00000000000000000002 mm, 0.00000000000000000001 mm, 0.000000000000000000007 mm, 0.000000000000000000005 mm, 0.000000000000000000003 mm, 0.000000000000000000002 mm, 0.000000000000000000001 mm, 0.0000000000000000000007 mm, 0.0000000000000000000005 mm, 0.0000000000000000000003 mm,os, of PEEP.

![Anesthesiology, V 82, No 4, Apr 1995](image)
be the result of pressure changes in the ventilator system.

Arterial blood was obtained from a cannula inserted via a femoral artery, with the tip positioned slightly above the aortic bifurcation. Blood gases were analyzed on a pH–blood gas analyzer (IL1306, Instrumentation Laboratories Spa., Milano, Italy).

In one set of experiments (n = 3) cardiac output was measured. A median sternotomy was performed and an ultrasonic flow probe (R6, Transonic Systems, Ithaca, NY) was applied on the ascending aorta and connected to a blood flow meter (T-201, Transonic Systems). Blood flow in the ascending aorta was used in a measure of cardiac output. In this set of experiments a PEEP of 10 cmH₂O was applied, and in 4 min during continuous measurement of cardiac output, and 10 min after the recovery from PEEP cardiac output was reduced to the same level as during PEEP by obstructing the pulmonary artery for 4 min by means of a strap around the artery. The latter procedure allowed gradual and stable reductions in cardiac output.

In another set of experiments (n = 7) bilateral vagotomy was performed by transecting the vagal nerves at the laryngeal level. Simultaneously, the depressor nerves were transected bilaterally. For simplicity, the combined nerve transection procedure is referred to as vagotony in the text. The effect of 10 cm PEEP before and after vagotomy was studied.

In all cases 45 min was allowed before experimentation to obtain stable circulatory conditions.

Statistical data are given as means ± SEM, and statistical significance was calculated by analysis of variance or two-tailed Student's t test for paired or unpaired observations. A P value < 0.05 was considered statistically significant.

Results

During a 30-min control period the measured parameters remained stable. Thus, mean arterial blood pressure was 89 ± 7 mmHg, heart rate 259 ± 27 beats·min⁻¹, arterial oxygen tension (PaO₂) 75 ± 12 mmHg, and the concentration of NO in exhaled air 19 ± 4 ppb (n = 13).

Introduction of PEEP elicited dose-dependent and highly reproducible increments in exhaled NO and in PaO₂. The increase in exhaled NO exhibited a biphasic pattern with an initial peak followed by a slow and partial reversal during the 4-min observation period at each level of PEEP (figs. 1 and 2). Thus, at a PEEP of

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...10 cmH₂O exhaled NO initially increased from 19 ± 4 to 30 ± 5 ppb (P < 0.001, n = 9) with a subsequent decrease to 27 ± 5 ppb (P < 0.005) at the end of a 4-min observation period. Simultaneously, PaO₂ increased from 75 ± 12 mmHg in the control situation to 105 ± 11 mmHg (P < 0.05) at a PEEP of 10 cmH₂O. In addition, PEEP induced dose-dependent reductions in systemic arterial blood pressure. Thus, at a PEEP of 10 cmH₂O mean arterial blood pressure decreased to 45 ± 7 mmHg (P < 0.01) (table 1). Heart rate was not significantly affected by PEEP.

Systemic treatment of the animals with the NO synthase inhibitor l-NAME (30 mg·kg⁻¹ intravenously) abolished NO in exhaled air during control conditions and during PEEP at all tested levels. In addition, l-NAME (30 mg·kg⁻¹ intravenously) increased mean arterial blood pressure from 78 ± 6 to 97 ± 5 mmHg. In the presence of l-NAME the PEEP-induced increments in PaO₂ were similar to those in the control situation. Thus, PaO₂ increased from 72 ± 8 to 98 ± 22 mmHg with PEEP (10 cmH₂O, n = 4).

In additional experiments the effect of vagotomy on the PEEP-induced increments in exhaled NO was studied. Consecutive applications of PEEP (10 cmH₂O) induced reproducible increases in NO. After bilateral vagotomy including bilateral transection of the depressor nerves the increase in exhaled NO in response to PEEP was significantly reduced (P < 0.01, n = 7) (fig. 3). Vagotomy did not alter the concentration of NO in exhaled air in the absence of PEEP.

To elucidate the importance of gross hemodynamic influences in the effect of PEEP (table 1) on NO in exhaled air the effect of PEEP on cardiac output was studied, and the effect of similar changes of cardiac output on exhaled NO was measured. In these experiments PEEP (10 cmH₂O) induced a reduction in cardiac output from 317 ± 36 to 235 ± 30 ml·min⁻¹ (P < 0.01, n = 3) and an increase in exhaled NO from 23 ± 6 to 30 ± 7 ppb (peak concentration, P < 0.05, n = 3). Reduction in cardiac output from 300 ± 67 to 223 ± 52 ml·min⁻¹ by partially obstructing the pulmonary artery (see methods) caused an insignificant increase in exhaled NO, from 23 ± 7 to 25 ± 6 (n = 3). The exhaled NO concentration during partial pulmonary artery obstruction was consistently and significantly less (P < 0.05) than exhaled NO during 10 cmH₂O PEEP.

Discussion

The results of this study demonstrate that PEEP induces an increase in the concentration of NO in exhaled air. This effect presumably involves vagal mechanisms and is only to a small extent due to hemodynamic changes.

PEEP levels have been shown to induce NO in exhaled air at moderate concentrations, suggesting that NO in exhaled air is a sensitive marker for pulmonary function. LOHMANN et al. have shown that PEEP levels below 10 cmH₂O do not increase NO production. However, the present study demonstrates that NO in exhaled air is a sensitive marker for pulmonary function.

Theoretical and physiological studies have shown that NO produced in the lung is a potent vasodilator. NO produced in the lung is a potent vasodilator. NO has also been shown to be a potent bronchodilator. The present study demonstrates that NO in exhaled air is a sensitive marker for pulmonary function.

Table 1. Effect of PEEP on Arterial Blood pH, PaO₂, and PaCO₂, Mean Arterial Blood Pressure (MAP), and Nitric Oxide Concentrations in Exhaled Air at Peak and Plateau Levels (n = 14).

<table>
<thead>
<tr>
<th>PEEP (cmH₂O)</th>
<th>pH</th>
<th>PaO₂ (mmHg)</th>
<th>PaCO₂ (mmHg)</th>
<th>MAP (mmHg)</th>
<th>NO Peak (ppb)</th>
<th>NO Plateau (ppb)</th>
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<tbody>
<tr>
<td>0</td>
<td>7.51 ± 0.04</td>
<td>75 ± 12</td>
<td>33 ± 2</td>
<td>89 ± 7</td>
<td>19 ± 4</td>
<td>19 ± 4</td>
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<tr>
<td>2.5</td>
<td>7.45 ± 0.05</td>
<td>76 ± 8</td>
<td>30 ± 1</td>
<td>76 ± 6</td>
<td>20 ± 5</td>
<td>19 ± 5</td>
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<tr>
<td>5</td>
<td>7.52 ± 0.05</td>
<td>97 ± 8</td>
<td>30 ± 2</td>
<td>68 ± 9</td>
<td>25 ± 4</td>
<td>23 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>7.52 ± 0.04</td>
<td>105 ± 11</td>
<td>31 ± 3</td>
<td>45 ± 7</td>
<td>30 ± 5</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>15</td>
<td>7.52 ± 0.12</td>
<td>94 ± 20</td>
<td>31 ± 7</td>
<td>40 ± 6</td>
<td>35 ± 7</td>
<td>31 ± 6</td>
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and is only to a minor degree influenced by the hemodynamic changes caused by PEEP.

PEEP levels frequently used in clinical practice were found to induce dose-dependent increments in exhaled NO in anesthetized rabbits. Considering the emerging knowledge of the considerable influence of NO on pulmonary function, the current observation may help in the understanding of the role of endogenous NO in lung function. Furthermore, the current observation opens the possibility that the increase in exhaled NO observed in response to agonist or antigen-induced bronchoconstriction is the result of increased tension in the airway wall or stretch of the lung parenchyma.

Theoretically, several mechanisms may explain the observed increase in NO with PEEP. First, PEEP increases functional residual capacity and reduces airway resistance. This effect likely increases the surface area of the respiratory epithelium exposed to air. Formation of NO in epithelium has been suggested by functional and morphologic studies. Provided that the NO detected in exhaled air is formed in the epithelium, such a mechanism could explain the increase in NO with PEEP. However, tidal volumes were kept constant in the current experiments, and on application of PEEP there was an initial peak in the concentration of NO in exhaled air, after which NO decreased to less markedly increased levels. This makes the increased airway wall surface area and its increased exposure to air unlikely as a single explanation for the increased NO in exhaled air. Second, PEEP causes hemodynamic alterations, with a decrease in cardiac output as a prominent effect. Complete obstruction of pulmonary blood flow induces profound increments in exhaled NO. However, when cardiac output was decreased by means of increasing pulmonary artery resistance in the current study, NO in exhaled air increased only moderately (or not at all), arguing against blood flow change as a reason for the increase in exhaled NO. In addition, changes in the tone of the pulmonary vasculature are not likely to explain the observed increments in exhaled NO, because pulmonary vasodilatation induced by infusion of adenosine elicits only a small effect on exhaled endogenous NO. The PEEP-induced increase in exhaled NO has also been observed during lung perfusion with constant flow conditions. Thus, changes in pulmonary blood circulation within the physiologic range seem less likely as an explanation for the effect of PEEP on NO in exhaled air, although contribution from such a mechanism can not be excluded. Finally, stretch of airways and lung parenchyma induced by PEEP is a conceivable stimulus for increased NO formation, analogous to the stretch-induced release of NO reported to occur in vascular endothelial cells. Involvement of a stretch-sensitive mechanism is supported in the current study by the observation that there was an initial peak in the concentration of NO in exhaled air, after which NO returned to concentrations closer to control on continued application of PEEP. Furthermore, the PEEP-induced increase in exhaled NO was attenuated by lesion of vagal and depressor nerves. Although the NO detected in exhaled air of rabbits likely is derived primarily from airway epithelium, pulmonary nerves of vagal origin have been suggested to release NO as an inhibitory neurotransmitter and in addition to the epithelium. NO synthase-like immunoreactivity has been observed in neurons within the lung. In accordance, it is possible that a stretch-sensitive and vagally dependent mechanism involves NO as a local effector mechanism. The type of cell that is stimulated by stretch cannot yet be identified, although epithelial and endothelial cells must be considered.

NAME, which inhibits NO formation and abolishes exhaled endogenous NO, did not affect the PEEP-induced increments in $P_{O_2}$. This observation and the small effect on exhaled NO of reductions in pulmonary blood flow support the suggestion that exhaled NO primarily reflects bronchial, rather than vascular NO production. An increase in exhaled amounts of NO has been observed during hyperventilation and exercise in humans, and the current observations may offer an initial insight into the mechanism of this effect.

In conclusion, PEEP elicited dose-dependent increments in exhaled NO. This effect may be attributed in part to a vagally mediated mechanism. Ongoing studies will reveal if this mechanism is present also in humans.

### References


### Table

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<thead>
<tr>
<th>NO Plateau (ppb)</th>
<th>19 ± 4</th>
<th>19 ± 5</th>
<th>23 ± 4</th>
<th>27 ± 5</th>
<th>31 ± 6</th>
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Microcirculatory Aspects of Awake Anesthesia

Wolfgang Funk, MD

Background: Blood vessels in the brain have a high density of nitric oxide donor molecules. When these molecules are released, nitric oxide is formed. Nitric oxide is a potent vasodilator and can reduce cerebral blood flow. It can also affect the microcirculation, which is important for drug delivery and tissue perfusion.

Methods: We studied the effect of nitroprusside (a nitric oxide donor) on cerebral blood flow and microcirculation in awake Syrian goldfish.

Results: Intravenous nitroprusside increased cerebral blood flow and caused a decrease in cerebral vascular resistance. The effect on microcirculation was variable, with some areas showing an increase and others a decrease in blood flow.

Conclusions: The effect of nitroprusside on the microcirculation is complex and may depend on the specific vascular area and its blood flow patterns.

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