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Inhalation of nitric oxide modulates methacholine-induced bronchoconstriction in the rabbit

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ABSTRACT: Nitric oxide (NO) accounts for the major effects of endothelium-derived relaxing factor. We investigated whether NO, added to the inspired gas, could exert a bronchodilatory action similar to the pulmonary vasodilation described when administering NO during lung vascular constriction.

New Zealand White rabbits were intubated and mechanically-ventilated with 30% oxygen during neuroleptanaesthesia. Methacholine (MCh) was nebulized at increasing concentrations from 0.5 to 4.0 mg·ml^{-1}, with or without inhalation of 80 parts per million (ppm) NO. The technique of rapid airway occlusion during constant-flow inflation was used for measuring respiratory mechanics, *i.e.* resistance and compliance of the respiratory system.

Methacholine nebulization without NO inhalation raised the resistance from 51±6 (mean±95% confidence interval) to 107±52 cmH_2O·l^{-1}·s at Mch 4 mg·ml^{-1}. During NO inhalation, nebulization of MCh showed no significant increase in resistance. Arterial oxygen tension (Pa_{O_2}) and compliance fell to the same extent during methacholine challenge, whether NO was inhaled or not. Closure of small airways may be a mechanism that causes the decrease in Pa_{O_2} and compliance observed.

This suggests that 80 ppm NO added to the inspired gas modulates the response in central airway tone to nebulized MCh in this rabbit model. However, it appears to have less effect on peripheral airways. *Eur Respir J., 1993, 6, 177–180.*

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Much interest has recently been focused on the pulmonary vasodilator effect of adding 5–80 parts per million (ppm) of nitric oxide (NO) to inspired gas in experimental [1, 2] and clinical pulmonary hypertension [3, 4]. In 1987 [5], NO was reported to be an important endothelium-derived relaxing factor (EDRF). Nitric oxide has a short half-life, of less than 10 s in tissue. It exerts its biological activity by binding to a haeme group in the enzyme guanylate cyclase, which in turn elevates the intracellular content of cyclic 3,5 guanosine monophosphate (cGMP) [6]. Nitric oxide is produced from L-arginine in the endothelial cells, and then diffuses to the subjacent layer of smooth muscle cells in the vascular wall [7]. In this way, a rapid and local regulation of vascular tone is achieved.

It has been proposed that the epithelium in the bronchial tree regulates airway tone in a similar fashion, by the release of a yet unidentified substance named epithelium-derived relaxing factor (EpDRF) [8, 9]. Some workers have presented data suggesting that NO may be different from EpDRF [10, 11]. A NO-releasing substance, sodium nitroprusside, has been demonstrated to cause bronchodilation in the sheep airway during experimental endotoxaemia [12]. In addition, other NO-releasing substances, such as nitrosothiols, dilate tracheal smooth muscles in tissue baths [13].

We wanted to discover whether NO had any effect on methacholine-induced bronchoconstriction in this model. We chose to study the bronchial effects of inhaled NO at the upper dose range for reported vascular effects, *i.e.* 80 ppm NO.

Materials and methods

Animal preparation

We studied eight New Zealand White rabbits of both sexes, with a mean body weight of 3.3±0.4 (mean±SD) kg. They were vaccinated against *Pasteurella* and *Bordetella,* and maintained on 75 g of high protein pellets a day and *ad libitum* water.

Premedication of 0.5 ml Hypnorm (Janssen, Belgium) [14] was given *i.m.* The marginal ear vein was used for *i.v.* injections, while the ear artery was used for blood sampling and pressure monitoring.

Before intubation, 1 ml Hypnorm *i.m.* and 5 mg diazepam *i.v.* were given. The larynx was sprayed with Lidocaine, 40 mg·ml^{-1}, which should not affect airway resistance [15]. Intubation was performed with a cuffed tube 3.0 (Sheridan, USA) and Wisconsin blade no.1 (Penlone, UK) on the laryngoscope. The rabbit was placed in a prone position on a heating pad to maintain normal body temperature. Artificial ventilation was given with a Siemens 900C ventilator (Siemens-Elema, Sweden), with an inspired oxygen fraction of 0.3, an inspiratory to expiratory time ratio of 1:2, tidal volume (VT) of 38 ml and a ventilatory frequency of about 30 breaths·min^{-1}. The ventilatory frequency was adjusted to keep the end-tidal CO_2 ($PETCO_2$) (Eliza Duo, Gambro-Engström AB, Sweden) at about 5 kPa, as verified with arterial blood gas analysis. Anaesthesia was maintained with *i.v* infusion of Hypnorm, 0.1–0.3 mg·kg^{-1}·h, and 2.5 mg diazepam *i.v.* as necessary. Muscle relaxant, pancuronium bromide, 0.2 mg, was given *i.v.* At the end of the experiment, the muscle paralysis was antagonized with neostigmine 0.15 mg and Robinul 0.03 mg (A.H Robins Co. Ltd, UK), and *i.v.* naloxone was given to counteract hypoventilation due to effects of residual Hypnorm.

Experimental procedure

After a 30 min stabilization period, a double VT was delivered to reopen any collapsed lung tissue. Inflation pressure (Pmax) limit was set to 20 cmH_2O.

Methacholine chloride (MCh), dissolved in distilled water 0.5–4 mg·ml^{-1}, was administered by means of an ultrasonic nebulizer (DeVilbiss, USA) fitted into the breathing circuit. The nebulizer generates droplets with a mass median diameter of 3 μ. Tests in rabbits have shown the aerosol to be distributed down the smallest airway [16]. The distilled water in which the MCh was dissolved should not exert any adverse effects. In another study soon to be published, in six rabbits nebulization with distilled water over 10 min had no effect on respiratory resistance (Rrs) or compliance (Crs).

Methacholine provocations were made with or without NO inhalation. The rabbits served as their own controls [17]: two weeks after the first experiment, those rabbits that had not inhaled NO received NO, and those rabbits that had previously inhaled NO, performed a MCh dose-response curve without NO inhalation. There were no significant differences in body weight or body temperature at the second experiment compared to the first one, two weeks earlier. The MCh was nebulized for one minute, with increasing concentration every 5 min. Nitric oxide 240 ppm in nitrogen (N_2) was obtained from AGA-Gas AB, Sweden. Nitric oxide 80 ppm was administered as a mixture with nitrogen and oxygen to the low-pressure gas supply inlet of the ventilator. A soda lime filter, to trap NO_2, was fitted into the breathing circuit on the inspiratory side. Tests of the delivery system were within 5% of the desired 80 ppm NO, and NO_2 concentration was less than 0.1 ppm over a 30 min period (chemiluminescence analysis, Tecan 502, Switzerland). Nitric oxide inhalation was started 5 min before MCh provocation and was maintained throughout the experiment. Data (mean arterial pressure (MAP), Pmax, $PETCO_2$, Rrs, Crs) were collected at baseline, after 5 min of NO inhalation and at each MCh concentration, with or without NO inhalation. Blood gases were determined at baseline and after 5 min of NO inhalation. In three rabbits, blood gases were also analysed at each MCh concentration.

Determination of respiratory mechanics

Measurements of Crs and Rrs were obtained using the technique of rapid airway occlusion during constant-flow inflation [18, 19]. Pressure and flow were measured in the ventilator on the inspiratory side, and fed into a computer for on-line signal processing (MacII Fx computer with LabView 2 software, USA). A mean value of two "inspiratory hold" manoeuvres was used for each point. Data were sampled within half a minute after completion of the MCh challenge. Volume and flow values were corrected for gas compression in tubings. Rrs is the difference between Pmax and the pressure 2 s after end-inspiratory pause, divided by the flow [20], with the endotracheal tube resistance of 28 $cmH_2O·l^{-1}·s$ subtracted. Crs was calculated as VT divided by the end inspiratory pressure minus the end-expiratory pressure.

Statistical analysis

Statistical analysis was performed using Students two-tailed test for paired data and the dose-response curves were tested with analysis of variance (ANOVA). Results are given as mean values ±95% confidence interval (CI) in the text and ± standard error of mean (SEM) in the figures. A value of $p<0.05$ was considered statistically significant.

Results

Effect of NO inhalation on baseline data

Baseline values of MAP, Pao_2, arterial carbon dioxide tension ($Paco_2$), Pmax, Rrs and Crs did not change after the two week interval (table 1). Nitric oxide inhalation *per se* did not significantly alter respiratory mechanics, blood gases or MAP (table 1, figs 1 and 2).

Effect of NO inhalation on methacholine-induced bronchoconstriction

Resistance. Methacholine nebulization resulted in a steady increase in Rrs from a baseline value of 51±6 (mean+CI) to 107±52 $cmH_2O·l^{-1}·s$ at the concentration of 4 mg·ml^{-1} ($p<0.05$). When NO was inhaled during MCh provocation, the increase did not reach significant levels, increasing from 51±8 to 72±26 $cmH_2O·l^{-1}·s$ at the concentration of 4 mg·ml^{-1}.

Table 1. – Baseline values (mean±95% confidence interval) for the experiments, and during nitric oxide inhalation before the methacholine provocation

	First baseline	Second baseline	NO inhalation
MAP mmHg	65±6	67±6	66±6
Pa_{O_2} kPa	25.9±1.4	26.7±3.2	23.0±6.0
Pa_{CO_2} kPa	4.6±0.4	4.3±0.4	4.7±0.3
Pmax cmH_2O	11.8±0.6	12.5±1.0	13.0±1.1
Rrs $cmH_2O \cdot l^{-1} \cdot s$	49±5	53±9	54±7
Crs $ml \cdot cmH_2O^{-1}$	4.3±0.6	4.5±0.6	4.1±0.5

MAP: mean arterial pressure; Pa_{CO_2}: arterial carbon dioxide; Pa_{O_2}: arterial oxygen tensions; Pmax: inflation pressure; Rrs, Crs and resistance: compliance of the respiratory system.

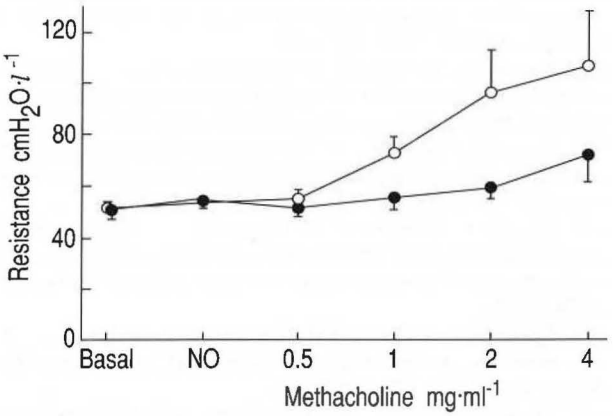

Fig. 1. – The methacholine (MCh) dose-response curve for resistance of the respiratory system (Rrs) was significantly modulated with inhalation of nitric oxide (NO) (●) (ANOVA $p<0.01$). The curve for MCh alone (○) increased significantly up to a concentration of 4 $mg \cdot ml^{-1}$ ($p<0.05$), while no significant increase was seen when NO was inhaled during MCh provocation. Data are mean±SEM. Anova: analysis of variance.

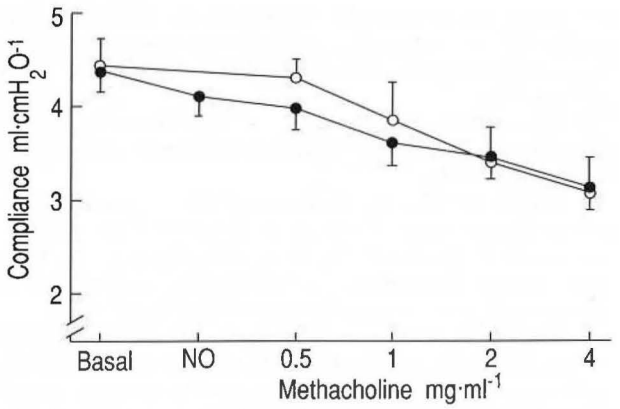

Fig. 2. – The methacholine (MCh) dose-response curve for compliance of the respiratory system (Crs), without (○) and with nitric oxide (NO) inhalation (●). There was no significant difference between the two curves. Data are mean±SEM.

Thus, the dose-response curve was significantly modulated by NO (ANOVA $p<0.01$) (fig. 1).

Compliance. Crs fell significantly in a dose-dependent manner from a baseline value of 4.4±0.7 to 3.1±0.9 $ml \cdot cmH_2O^{-1}$ at a MCh concentration of 4 $mg \cdot ml^{-1}$ ($p<0.01$). With NO inhalation during MCh challenge, the Crs fell from a baseline value of 4.4±0.5, to the same extent as without NO, *i.e.* 3.1±0.6 $ml \cdot cmH_2O^{-1}$ at a MCh concentration of 4 $mg \cdot ml^{-1}$ ($p<0.01$). Thus, there was no difference between the two dose-response curves (fig. 2).

Arterial blood gases. In the three rabbits, in which blood gases were analysed at each MCh dose with or without NO inhalation, the Pa_{O_2} fell, while Pa_{CO_2} was not affected. The decrease was 7 kPa for MCh only (25%) and 10 kPa when NO was inhaled (34%) during the provocation test. There was a good correlation between Pa_{O_2} and compliance ($r=0.76$, $p<0.001$) (fig. 3). However, there was no correlation between Pa_{O_2} and resistance.

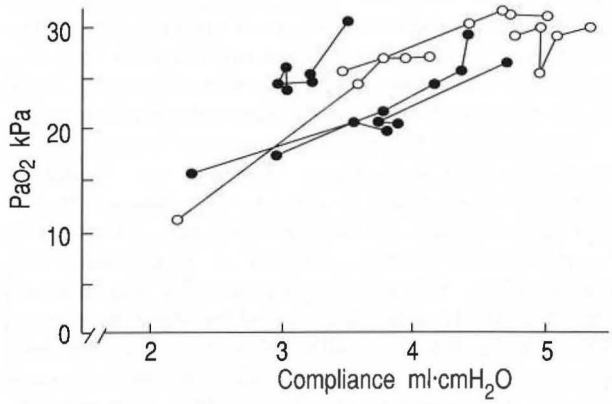

Fig. 3. – Plot of arterial oxygen tension (Pa_{O_2}) against compliance of the respiratory system. Data from three rabbits during baseline and provocation with increasing concentrations of methacholine (MCh), without (○) or with (●) nitric oxide inhalation. Data from each individual rabbit obtained during one experiment are connected by lines. Note the similar decreases in Pa_{O_2} and compliance with increasing MCh concentration, without and with nitric oxide.

Discussion

At the given concentration (80 ppm), NO exerted no bronchodilatory effects during non-constricted conditions and it had no adverse effects on gas exchange. During MCh challenge, inhalation of NO protected the rabbit from bronchoconstriction. However, inhaled NO did not counterbalance the fall in compliance and arterial oxygenation induced by MCh. Similar or lower concentrations of NO were sufficient to totally abolish hypoxic pulmonary vasoconstriction induced by breathing 12% O_2 in sheep (40–80 ppm) [1], and in humans (10 ppm) [21], and also thromboxane-mediated vasoconstriction in sheep (5–80 ppm) [2]. This suggests that at least part of the NO must have reached the alveoli, to be able to act on the peripheral vascular system.

The protective effect of inhaled NO on resistance during MCh challenge, and the simultaneous decrease in compliance and arterial oxygenation, may be explained as follows. The recording of resistance reflects to a major extent the behaviour of the central airways, whereas the decrease in oxygenation and compliance are more probably due to changes in the

periphery of the lung. It may thus be that NO exerted its effect primarily on the central airways and less on the small airways, so that an increase in peripheral airway constriction by MCh still occurred during NO inhalation. Peripheral bronchoconstriction may cause airway closure and airtrapping, impeding oxygenation (reduced Pa_{O_2}) and making the lung stiffer by regional hypoventilation and overinflation (decreased compliance). Further support for this hypothesis is given by the lack of correlation between resistance and Pa_{O_2} and the presence of a correlation between compliance and Pa_{O_2}. *In vitro* experiments on canine airways have also demonstrated a relaxing effect on the trachea by exposure to NO, and much weaker effects on bronchi [22]. Recently, DUPUY *et al.* [23] reported on the effects of inhaled NO in mechanically-ventilated, open chest guinea-pigs during continuous intravenous infusion of methacholine. They found a dose-response relationship between NO concentration and bronchodilation, with increasing effect up to 300 ppm NO, the maximum concentration tested.

No data have yet been presented on airway effects of inhaled NO in humans. A report on ventilation/perfusion relationships in patients with chronic obstructive lung disease who inhaled NO, showed increased ventilation to poorly perfused or unperfused lung regions [24]. The authors proposed that this indicated regional airway dilatation caused by the inhalation of NO. Our group has continued with a study on asthmatics. The first two patients have shown a decrease in airway resistance of 25 and 28%, respectively, after NO inhalation. However, both patients responded with larger reduction in airway resistance on $ß_2$-agonist after the termination of NO inhalation (58 and 53%, respectively). More patients have to be studied before any conclusions can be drawn.

In summary, inhalation of 80 ppm nitric oxide counter balanced an increase in the respiratory resistance of the rabbit during methacholine provocation. It is hypothesized that NO exerts its effect on the large airways.

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