Airway response to deep inspiration: role of nitric oxide

R.H. Brown*, W. Mitzner[#]

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ABSTRACT: Deep inspirations (DI) have been shown to have both bronchoprotective and bronchodilator effects in healthy subjects. The bronchodilator effects of a DI appear to be impaired in asthmatics compared with healthy subjects. This study investigated the role of nitric oxide (NO) in the bronchodilator role of a DI.

In five anaesthetised and ventilated dogs, high-resolution computed tomography was used to measure the changes in airway size after a small (25 cmH$_2$O) and large (45 cmH$_2$O) DI before and after administering N^G-nitro-L-arginine methyl ester to block NO synthesis.

The depth of the inspiratory manoeuvre during a deep inspiration determined the subsequent qualitative behaviour of the airway response. Inflation to relatively high pressure resulted in airway dilation, whereas one to lower pressure leads to airway constriction. When N^G-nitro-L-arginine methyl ester was administered, both a large and a small deep inspiration resulted in subsequent airway constriction. These results support the idea that nitric oxide may be a potential bronchoprotective agent in the airways.

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*Dept of Anaesthesiology and Critical Care Medicine and [#]Dept of Environmental Health Sciences/Division of Physiology, The Johns Hopkins University, Baltimore, MD, USA.

Correspondence: R.H. Brown, Physiology, Room 7006, Johns Hopkins University, Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA.
Fax: 1 4109550299
E-mail: rbrown2@jhem.jhmi.edu

Deep inspirations (DI) have been shown to have both bronchoprotective and bronchodilator effects in healthy subjects [1, 2]. However, the bronchodilator effects of a DI appear to be impaired in asthmatics compared with healthy subjects [2]. In addition, separate experiments by BRUSASCO *et al.* [3] and BROWN *et al.* [4] suggest that there are intrinsic differences between the responses to lung inflation in airways from asthmatic and normal subjects. Healthy normal subjects demonstrated bronchodilation after the DI, whereas asthmatic subjects showed further bronchoconstriction after the DI manoeuvre [4].

When considering the effects of a DI, an implicit assumption is that all of the airways are distending to their maximal size at total lung capacity (TLC). However, it was shown previously in a canine model that the degree of static distension of individual airways was highly variable both within and among animals [5]. Fully relaxed airways were quite distensible at low lung volume, but quickly reached a maximal size with no further distension up to TLC. With smooth muscle tone, the airways showed variable degrees of dilation with lung inflation, but full distension of even moderately constricted airways was rarely achieved, even at TLC [5].

In assessing the response of airways to DI, it would therefore seem important to use a consistent and well-defined lung volume history. However, in human subjects it is not routine to measure transpulmonary pressure and often even the duration of the DI is not well characterised. In a recent study, the authors demonstrated that the length of time at TLC could alter the qualitative response of the airways following the DI [6]. Furthermore, the degree of lung inflation during the DI was also shown to affect the subsequent airway response [7]. If the peak inflation pressure during a DI was large (45 cmH$_2$O), the airways distended and then returned slowly, asymptotically approaching the pre-DI size. However, if the peak inflation pressure was small ($\leqslant$35 cmH$_2$O) the airways still dilated during the DI, but recovered to a size that was up to 30% smaller than the pre-DI level. No mechanisms were investigated in that study, but the authors speculated that perhaps a bronchoconstrictor or bronchodilator was released in response to small or large stretches, respectively. In the present study the authors investigated a possible role for nitric oxide (NO) in this differential response to the level of lung inflation during a DI.

Methods

The study protocol was approved by the Johns Hopkins Animal Care and Use Committee. Five dogs weighing ~20 kg were anaesthetised with thiopental (15 mg·kg^{-1} induction dose followed by 10 mg·kg^{-1}·h^{-1} *i.v.* maintenance dose). After induction of anaesthesia, the dogs were paralysed with 0.5 mg·kg^{-1} of succinylcholine, with occasional supplemental doses as required to ensure no respiratory motion during imaging. Following endotracheal intubation with an endotracheal tube (inside diameter 8.0 mm), the dogs were placed in a supine position and their lungs were ventilated with room air using a volume-cycled ventilator (Harvard Apparatus, Millus, MA, USA) at a tidal volume of 15 mL·kg^{-1} and a rate of 18 breaths·min^{-1}. A stable depth of anaesthesia was maintained by monitoring heart rate changes and eyelash reflex.

Imaging and analysis of airways

High-resolution computed tomography (HRCT) scans were obtained with a Somatom Volume Zoom scanner (Siemens, Iselin, NJ, USA), using a spiral mode to acquire 50 computer tomography images during a 8 s breath hold (apnoea) at 137 kVp and 165 mA. The images were reconstructed in slices of 1 mm and a $512{\times}512$ matrix, using a 125 mm field of view and a high spatial frequency (resolution) algorithm that enhanced edge detection at a window level of -450 Hounsfield units (HU) and a window width of 1,350 HU. These settings have been shown to provide an accurate measurement of lumenal size to as small as 0.5 mm in diameter [8, 9]. For repeated airway measurements in a given dog within each experimental protocol, adjacent anatomic landmarks, such as airway or vascular branching points, were defined and used to measure the airway size at the same anatomic cross sections.

The HRCT images were analysed using the airway analysis module of the Volumetric Image and Display Analysis image analysis software package (University of Iowa, IA, USA) as previously described and validated [5, 10]. The HRCT images were transferred to a UNIX-based Sun workstation (Sun Microsystems, Santa Clara, CA, USA). An initial isocontour was drawn within each airway lumen and the software program then automatically located the perimeter of the airway lumen by sending out rays in a spoke-wheel fashion to a predesignated pixel intensity level that defines the lumenal edge of the airway wall. Intra- and inter-observer accuracy and variability of the software program using this HRCT technique in phantoms, consisting of rigid tubes to measure known areas, has been shown previously by the authors [9] and by others [10] to be highly resistant to operator bias.

HRCT does not allow differentiation of the vessel wall from blood. Since the vessel walls are thin compared with the overall size of the vessel, total vessel area was measured to assess changes in vascular volume using the software package noted above. The operator drew a rough isocontour estimate of the total vessel area and the software program automatically located a precise isocontour of the vessel external perimeter as described for the airway lumenal area above. In each dog, 20–33 vessels of 1.2–6.0 mm diameter, were matched and measured prior to the DI.

Protocol

Dogs were anaesthetised and ventilated as described above. To standardise lung volume history, the dogs were initially given a DI of both lungs to 35 cmH$_2$O for 5 s. Then a stable state of airway tone was induced with a continuous *i.v.* infusion of 67 µg·min^{-1} methacholine (MCh; Sigma Chemical, St Louis, MO, USA). The tubing from the ventilator to the endotracheal tube of the animals had an added large bore Y-connector. One branch of the Y went to the ventilator and the other branch was connected to a constant pressure source set at either 25 or 45 cmH$_2$O. This source consisted of an underwater overflow fed by a line from a high-flow oxygen supply. At the start of scanning, the ventilator was simultaneously shut off, a solenoid valve to the ventilator was closed and another solenoid to the pressure source was opened to the dog for either 10 s (for 25 cmH$_2$O pressure) or 30 s (for 45 cmH$_2$O pressure). Solenoids were then switched to suddenly expose the trachea to atmospheric pressure and the scans were acquired. Scanning was performed immediately after the DI (~4 s) and at 30, 60, 90, 120 and 300 s after the DI. After each scan set was acquired (~20 s of apnoea), the ventilation was resumed. The manoeuvre was then repeated, setting the constant pressure source to the alternate pressure. The 25 cmH$_2$O DI was performed first, followed by the 45 cmH$_2$O manoeuvre, with ⩾10 min between manoeuvres. The lung inflation manoeuvres were then repeated after N^G-nitro-L-arginine methyl ester ((L-NAME) Sigma Chemical) was administered by *i.v.* infusion (50 mg·kg^{-1}). To measure the size of the completely relaxed airways, the dogs received 0.2 mg·kg^{-1} of atropine at the end of the experimental protocols. Previous work has demonstrated that this dose of atropine abolishes all cholinergic smooth muscle tone in dogs [5].

Analysis

The initial airway area prior to the DI was defined as 100%. The airway lumenal areas were expressed as a per cent of this initial area (dependent variable). Each airway in each dog served as its own control. Generalised analysis of variance was used to compare the airway size between the two DI pressures before and after the administration of L-NAME, and at each time point (independent variables). Fisher's protected least significant difference, Scheffe and Bonnferroni/Dunn corrections for multiple pairwise comparisons were used. In addition, paired t-tests were used to compare the changes in vessel size before and after L-NAME. $p \leqslant 0.05$ was considered significant.

Results

In each dog, 23–39 airways, ranging 1.2–8.5 mm in diameter, were matched and measured during MCh infusion. MCh infusion at the chosen rate caused stable airway constriction to 37±1% (mean±SEM) of their completely relaxed size. All subsequent measurements were referenced to their baseline (pre-DI) size with MCh that was defined as 100%. At the first (4 s) measurement after the DI, the mean±SEM airway area as a per cent of baseline area prior to the DI was 133±3 and 180±4% of pre-DI for the 25 and 45 cmH$_2$O DIs, respectively. The airway area after the 45 cmH$_2$O DI was significantly greater than the airway area after 25 cmH$_2$O DI (p<0.0001; fig. 1). During the 5-min interval following the DI, the airway size decreased in a quasi-exponential manner. However, there was a qualitative difference in the response of the airways to the DI depending
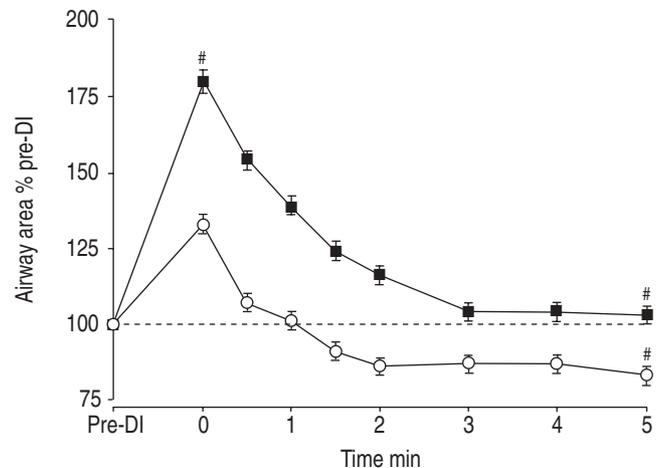

Fig. 1. – Per cent change in mean±SEM airway area immediately after a deep inspiration (DI) to a peak inflation pressure of 25 cmH$_2$O (○) and 45 cmH$_2$O (■). #: p<0.0001.

on the size of the DI. For the larger DI (45 cmH$_2$O), the airway size remained above the baseline airway area for the entire measurement period. At 5 min, the airway area was 103±3% of pre-DI, which was not different from the pre-DI value (p=0.26; fig. 1). In contrast, for the smaller DI (25 cmH$_2$O), the airways actively contracted to a smaller area than at the pre-DI baseline. At 5 min, the airway area was 83±3% of the pre-DI airway area (p<0.0001; fig. 1). The airway size at 5 min was also significantly different between both pressures (p<0.0001).

After administration of L-NAME, the vessels′ area decreased significantly by 21±1% compared with baseline (p<0.0001), and the airways dilated significantly from 37±1 to 60±1% (p<0.0001 compared with baseline airway size prior to L-NAME) of their completely relaxed size.

At the first (4 s) measurement after the DI, the mean airway area as a per cent of post-L-NAME baseline area prior to the DI was 118±3 and 125±7% of pre-DI for the 25 and 45 cmH$_2$O DIs, respectively and these values were not significantly different (p=0.37, fig. 2). During the 5-min interval following the DI, the airway size decayed to a value smaller than the pre-DI size, but, unlike the situation prior to L-NAME, there was no qualitative difference in the response of the airways to the DI depending on the size of the DI. For both the 25 and the 45 cmH$_2$O DIs, the airways were actively contracted at 5 min to 94±2 and 81±2% of pre-DI airway area for the 25 (p=0.0001 compared with pre DI) and 45 cmH$_2$O DIs, respectively (p<0.0001 compared with pre-DI, fig. 2).

Discussion

In previous studies, the authors have demonstrated that the manner in which a DI was performed could determine whether the airways responded with a subsequent constriction or dilation. If the DI was performed in a brief period to a relatively small inflation pressure, then airway constriction was observed. However, if the lungs were expanded to a higher pressure for a longer period of time, then the airways remained dilated for many minutes after the manoeuvre [6, 7]. This behaviour may result from two distinct processes, one being a passive viscoelastic stretching of the airway muscle that takes a long time to recover, and the other reflecting some degree of active constriction in response to the lung
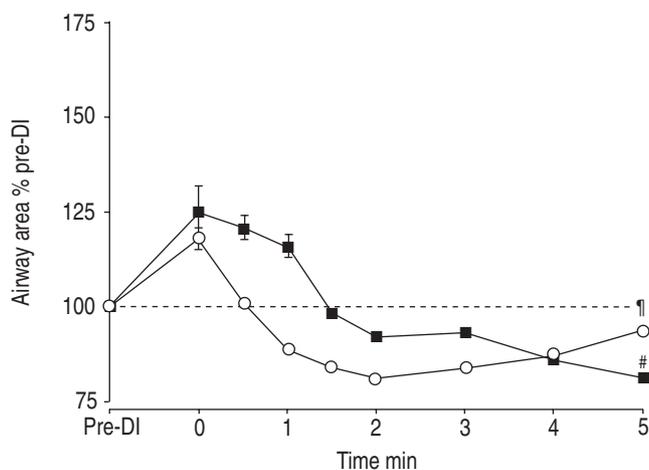

Fig. 2. – Per cent change in mean±SEM airway area immediately after a deep inspiration (DI) to a peak inflation pressure of 25 cmH$_2$O (○) and 45 cmH$_2$O (■) after *i.v.* administration of N^G-nitro-L-arginine methyl ester . $^\#$: p<0.0001; ¶: p=0.0001.

inflation. The present study was designed to test whether NO may be important in the response of the airways to this deep inspiratory manoeuvre. To this end, the experiment compared the airway response to DIs performed at both a large and small maximal inflation pressure.

The current results not only confirmed previous observations by the authors showing that the extent of lung inflation during a DI had a major effect on the subsequent airway size, but also clearly showed that NO appeared to play a role in this response. A large DI initially caused airway dilation and the airways stayed slightly dilated even at 5 min post-DI. However, a small DI caused less initial airway distension and subsequent airway constriction over time. After administering L-NAME to the dogs, the large DI resulted in smaller peak airway distension and also caused a subsequent airway constriction, similar to that normally observed only with the smaller DI inflation. These results suggested that a sudden large inflation of the lung may normally result in release of NO that acutely dilates airway smooth muscle. If this increase in NO was blocked, an intrinsic constrictor response was unmasked. Smaller lung inflations may be insufficient to cause this release of NO.

NO is a ubiquitous molecule throughout the body that serves several biological functions. One well-documented function of NO is as a vasodilator [11, 12], and although there is some evidence that NO can also act as a bronchodilator, this remains controversial [13–17]. NO is synthesised from L-arginine by one of three NO synthase enzymes, which then diffuses into cells and acts through a guanylcyclase mechanism to relax smooth muscle. L-NAME is an NO synthase inhibitor and administration of L-NAME has been widely used to block NO synthesis *in vivo*. Thus, *i.v.* infusion of L-NAME in dogs should block the production of NO in the airways and pulmonary vessels causing constriction. Although under baseline conditions a 21% constriction in the pulmonary vessels was observed, the average dilation of the airways was 62%. The reason for this unexpected behaviour of the airways is unclear, but it was consistently found in all animals. One possible contributing factor was that the constricted vessels led to a mechanical dilation of the airways *via* an interdependence mechanism. In previous work studying the effect of vascular engorgement on airways, the authors observed a substantial narrowing of the airways when the vessels were engorged [18]. Although the authors would predict the opposite effect on airways when the vessels are constricted, it is unlikely that the magnitude of this effect could account for the entire increase in airway size. Another contributing factor may be a decrease in cardiac output caused by the pulmonary vascular constriction, leading to a decreased venous return. Such a decrease in systemic blood flow to the airways may lead to a decrease in the delivery of MCh to the airways. This possibility is supported by the work of WAGNER and MITZNER [19], using a sheep model of airway constriction. When infusing MCh at a constant concentration into the airways through the bronchial circulation, a decrease in bronchial blood flow caused a decrease in airway resistance [19]. Any decrease in cardiac output in animals in this study would likely be reflected in a proportional decrease in bronchial blood flow and thereby decrease the available MCh in the airway wall. This effect may also be exacerbated by a direct effect of blocking NO synthesis on the bronchial vasculature.

Of even more relevance than the baseline changes after the administration of L-NAME, were the airway responses to DI. After L-NAME, the post-DI airway constriction normally observed only after a small DI now also occurred even after the large DI. Furthermore, the peak recorded distension of the airways after both small and large DIs was reduced compared with baseline values. This latter observation may

result from the airways being more dilated after the L-NAME. That is, as the airways enlarge and get closer to their maximal size, their ability to dilate, given the same distending force, should decrease. While this mechanism can partially explain why the airways dilate less during the DI manoeuvre, the question still remains as to why the airways now constrict following the DI.

One explanation for this post-DI constriction, suggested in a previous study by the authors [7], was that a contractile mediator is being released in a small stretch of the lung and airways. Such a contractile stimulus would be present with both large and small inflations, but with large inflations, the larger physical distension would be more than sufficient to counterbalance this constrictor effect. A specific mediator was not proposed in this previous work. However, recently an idea was proposed that NO may normally be released during a DI to prevent airway constriction [20]. It was also suggested that a DI may lead to bronchoprotection because of the release of NO to minimise subsequent airway constriction to MCh challenge. The result could be consistent with this hypothesis, if it assumed that contracted airways have a myogenic response to stretch [21]. A small DI may be insufficient to release enough NO to prevent the subsequent broncho-constriction caused by the myogenic stretch response. With a large DI, increased amounts of NO are released, which counteracted the smooth airway constriction, allowing the airways to remain dilated. After L-NAME, the peak distension of the airways was similar with both pressures, and since there was no NO released, the airways constricted in both cases. This explanation is speculative at this time, both because NO in the airways was not measured and because there is scant evidence that airway smooth muscle shows an active myogenic constriction to stretch *in vivo*. However, previous *in vitro* work with canine bronchial segments clearly demonstrated that contraction can occur in response to airway distension [22], but the extent of this effect *in vivo* has not been evaluated.

The levels of peak inflation used in the current study were well within the range that can be obtained in humans. Although pleural pressure was not measured, the approximate transpulmonary pressures can be estimated from a previous study where such measurements were performed [5]. In that work, when the airway pressure was 36 cmH_2O, the transpulmonary pressure was 23 cmH_2O. With a simple linear extrapolation, it was estimated that the transpulmonary pressures at each of the airway pressures used in this study were 17 and 30 cmH_2O.

There is little relevant experimental evidence in the literature that has attempted to address the size of DI on subsequent airway tone. Several current clinical investigations have focused on single or repetitive DIs as a means of preventing or reversing bronchoconstriction [1–3, 23]. In general, the nature of the DIs in most clinical studies is not explicitly specified, but they are usually brief and sigh-like, typically in the order of only a few seconds. How these results relate to the paradoxical airway narrowing sometimes seen in asthmatics following a DI [3, 4, 24–30] remains uncertain. Perhaps asthmatic airways are stretched too little during the manoeuvre and behave like the low pressure groups in the present work. BROWN *et al.* [4] have studied the ability of a DI to distend the airways of normal healthy volunteers compared with mild asthmatics subjects at baseline and after increased tone [4]. A DI distended the airways of healthy and asthmatic subjects to a similar extent. Furthermore, it was observed that after constriction had already been induced by MCh, a deep inspiration resulted in bronchodilation in the healthy subjects, but further bronchoconstriction in the asthmatics, consistent with a loss of or insensitivity to a bronchodilator, such as NO.

In summary, it has been confirmed that the size of the depth of the inspiratory manoeuvre during a deep inspiration determined the subsequent qualitative behaviour of the airway response. Inflation to relatively high pressure resulted in airway dilation, whereas one to lower pressure led to airway constriction. Furthermore, when N^G-nitro-L-arginine methyl ester was administered to block nitric oxide, both a large and a small deep inspiration led to subsequent airway constriction. These results support the idea of nitric oxide as a potential bronchoprotective agent in the airways.

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