Bronchoprotective and bronchodilatory effects of deep inspiration in rabbits subjected to bronchial challenge

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Received 8 March 2001; accepted in final form 30 July 2001

Gunst, S. J., X. Shen, R. Ramchandani, and R. S. Tepper. Bronchoprotective and bronchodilatory effects of deep inspiration in rabbits subjected to bronchial challenge. J Appl Physiol 91: 2511–2516, 2001.—The effect of deep inspiration (DI) on airway responsiveness differs in asthmatic and normal human subjects. The mechanism for the effects of DI on airway responsiveness in vivo has not been identified. To elucidate potential mechanisms, we compared the effects of DI imposed before or during induced bronchoconstriction on the airway response to methacholine (MCh) in rabbits. The changes in airway resistance in response to intravenous MCh were continuously monitored. DI depressed the maximum response to MCh when imposed before or during the MCh challenge; however, the inhibitory effect of DI was greater when imposed during bronchoconstriction. Because immature rabbits have greater airway reactivity than mature rabbits, we compared the effects of DI on their airway responses. No differences were observed. Our results suggest that the mechanisms by which DI inhibits airway responsiveness do not depend on prior activation of airway smooth muscle (ASM). These results are consistent with the possibility that reorganization of the contractile apparatus caused by stretch of ASM during DI contributes to depression of the airway response.

IN HEALTHY SUBJECTS WITH INDUCED bronchoconstriction, a deep inspiration (DI) attenuates the degree of airway narrowing (18). In contrast, many asthmatic subjects demonstrate either no change or a slight decrease in airway resistance (Raw) after DI (1, 2, 33). Malmberg and co-workers (16) reported that, in healthy human subjects, DI before methacholine (MCh) inhalation also attenuates the subsequent airway response to the challenge. Other investigators have also described a bronchoprotective effect of DI in human subjects (12, 14).

Many of the effects of DI on airway responsiveness observed in vivo can be mimicked by imposing length changes on isolated airway smooth muscle strips in vitro, suggesting that the mechanisms for these effects may be intrinsic to the properties of airway smooth muscle. When the length of airway smooth muscle is decreased during contractile activation, force redevelopment and shortening velocity at the shorter length are depressed below the values obtained when the contractions are initiated at the shorter length (6, 11, 17). If a similar decrease in tracheal muscle length is imposed immediately before contractile stimulation, force development in response to the subsequent stimulus is also depressed (6, 11, 17). In addition, length oscillation of tracheal muscle before contractile stimulation also inhibits force development (32).

Several hypotheses have been proposed to account for the effects of length perturbations on airway muscle contractility in vitro. The oscillation or shortening of activated smooth muscle over a large length range would be expected to detach cross bridges (3, 24). If the activation state of the muscle declines and latches bridges develop during the period of contraction, force might not redevelop fully after a length perturbation (4, 5, 15, 25). However, this mechanism does not account for the depression of contractility that is observed when a length perturbation is imposed on the muscle before the administration of a contractile stimulus. In tracheal muscle strips in vitro, a length step or length oscillation depresses subsequent force development even when no active basal tone is present (6, 8, 11, 17, 32). Furthermore, the depression of force development after the imposition of a shortening step either before or after the initiation of contraction is not associated with a depression of myosin light chain phosphorylation, suggesting that shortening does not induce a depression of the activation state of the contractile apparatus (17).

We and other investigators have hypothesized that a plasticity of the cellular organization of the contractile apparatus in smooth muscle may contribute to the effects of length perturbations on airway muscle contractility (8, 11, 23, 24, 32). By this hypothesis, the organization of the contractile apparatus is modified by the cell to adapt to changes in cell shape that occur when muscle length is changed, enabling smooth muscle cell contractility to be optimized to its physical environment. When a change in length is imposed on the cells immediately before contractile activation, the organization of the contractile apparatus cannot adjust...
to the change in cell length, resulting in a depression of contractility.

The mechanism for the effects of DI on airway responsiveness in vivo may differ when the DI is imposed before bronchoconstriction vs. during bronchoconstriction. In the present study, we continuously monitored the Raw in response to MCh after DI in rabbits in vivo after imposing a DI either before or during induced bronchoconstriction. Although it has been suggested that the bronchoprotective effect of DI might result from the disruption of baseline tone, there are currently no data to support or refute this hypothesis. Because rabbits do not exhibit significant basal airway tone (13, 26), the use of rabbits for these studies allowed us to evaluate whether cross-bridge detachment is the primary mechanism for the bronchoprotective effect of DI. If the depressive effect of DI on airway responsiveness is primarily due to the disruption or modulation of cross-bridge attachments, a DI before bronchoconstriction should not significantly affect the response to a subsequent MCh challenge. However, if the effect of DI on airway responsiveness is due to a mechanism that does not require cross-bridge activation, the effect of DI on airway responsiveness might be similar when the DI is imposed before or during induced bronchoconstriction. It has been suggested that a DI imposed during bronchoconstriction does not depress but merely delays the airway response to MCh (25). By monitoring the time course of the airway response continuously, we are able to follow the change in Raw that occurs in response to each maneuver and identify the maximum response.

We have previously demonstrated that the airway reactivity of immature rabbits to MCh is greater than for mature rabbits. Tidal ventilation depresses the magnitude of the airway response to intravenous MCh in vivo in proportion to the magnitude of the tidal volume (23). We speculated that the difference in airway reactivity between mature and immature rabbits might therefore be related to a difference in the effect of lung volume maneuvers on airway responsiveness. An additional objective of the present study was, therefore, to compare the effects of DI on the airway response to MCh in immature and mature rabbits.

METHODS

Experimental Preparation

Eleven mature (5–6 mo, 2.5–3.0 kg) and twelve immature (4–6 wk, 0.4–0.6 kg) New Zealand White rabbits were evaluated. The animals were anesthetized with intravenous pentobarbital sodium (50 mg/kg). After tracheotomy, an appropriately sized tube was inserted and securely tied in place to prevent air leaks. Animals were mechanically ventilated (model 683, Harvard) with a tidal volume of 6 ml/kg at a frequency of 40 breaths/min. The expiration port of the ventilator was connected to a water column that maintained positive end-expiratory pressure (PEEP) at 4 cmH2O. A jugular venous catheter was inserted to administer additional anesthetic, normal saline, and MCh. The abdominal and thoracic cavities were widely opened, and a warming pad was used to prevent cooling of the animal.

Tracheal pressure (Ptr) was measured with a piezoresistive pressure transducer (model 8507C0-2, Endevo, San Juan Capistrano, CA), and tracheal flow was measured with a screen pneumotachometer (model 8410A, Hans Rudolph, Kansas City, MO) and a differential pressure transducer (±2.25 cmH2O; model MP45, Validyne, Northridge, CA) attached to the tracheotomy tube. Because the chest was open, changes in Ptr could be used to assess changes in transpulmonary pressure. Analog signals of flow and pressure were analog filtered above 50 Hz, amplified, and digitized at 100 samples/s (model DT2801-A, Data Translation Marlborough, MA). Digital signals were stored in an IBM-compatible personal computer (model 2000, Gateway, North Sioux, SD) using data acquisition software (RHT Infodat, Montreal, PQ, Canada).

Raw was assessed from changes in pressure and flow at the airway opening produced by very-small-volume (0.2–0.3 ml/kg) forced oscillation at 6 Hz. These oscillations were generated using a small piston attached to a linear motor that was in parallel with the ventilator. Raw was then calculated with a linear regression technique to fit pressure and flow (V˙) signals to Eq. 1

$$\text{Ptr}(t) = \text{Raw}(t) \cdot \dot{V}(t) = (t) + K(t)$$

where $K$ is a constant that absorbs any small offsets in the mean value of Ptr, and $t$ is time. By use of commercial software (RHT Infodat), Raw was calculated by recursive least squares with a memory time constant of 0.5 s.

Raw was monitored at PEEP of 4 cmH2O in a closed-circuit constant-volume system. During the measurements of Raw, there was no fresh gas entering the circuit.

Protocol for evaluating effect of DI after MCh administration on the airway response. Each animal was challenged with five doses of intravenous MCh (0.005 mg/kg; Fig. 1). The first, the third, and the last (fifth) challenges were obtained under static conditions (no DI). For these three challenges, the ventilator was turned off, small-volume oscillations were initiated to monitor changes in Raw, and then a bolus intravenous dose of MCh was administered. After 50 s, the small-volume oscillations were stopped and mechanical ventilation was resumed with fresh gas entering the circuit. Immedi-

![Fig. 1. Changes in airway resistance (Raw) with time during successive challenges with intravenous methacholine (MCh; 0.005 mg/kg) in a representative rabbit. Responses to challenges 1, 3, and 5 (solid lines) were obtained under static conditions (no deep inspirations (DI)), and responses to challenges 2 and 4 (shadowed lines) were obtained when a single DI was performed immediately after the onset of the response to the MCh.](image-url)
ately after administration of the MCh bolus for challenges 2 and 4, a syringe was used to transiently increase lung volume to near total lung capacity. The duration of the DI (inflation and deflation) was 2–3 s with no end-inspiratory pause; the peak inspiratory pressures were between 15 and 20 cmH2O. For each challenge, after the maximum response to MCh was attained, the rabbit was given three DIs to total lung volume and then ventilated until Raw returned to baseline, ~20 min.

After the completion of the above protocol, two challenges were performed using 0.01 mg/kg MCh. A DI was performed during one of these challenges in random sequence.

Protocol for evaluating effect of DI before MCh administration on the airway response. As described above, each animal underwent five intravenous MCh challenges (0.005 mg/kg) at a PEEP of 4 cmH2O in a closed-circuit constant-volume system (see Fig. 4). The first, the third, and the last (fifth) challenges were obtained under static conditions (no DI). For MCh challenges 2 and 4, lung volume was cycled to total lung capacity four times using a syringe immediately before administration of the intravenous MCh. The duration of each DI (inflation and deflation) was 2–3 s with no end-inspiratory pause; the peak inspiratory pressures were between 15 and 20 cmH2O.

In a separate group of four rabbits, we tested the absence of airway tone under basal conditions by administering intravenous atropine (2 mg/kg) or by vagotomy.

**Data Analysis**

A value for maximum Raw was calculated from measurements of the time course of changes in Raw, by averaging Raw values from 2.5 s before to 2.5 s after the peak value of Raw. To control for the effects of time and the sequence of the MCh challenges, the maximum Raw under static conditions (no DI) was obtained by averaging responses to the first, third, and fifth MCh challenges. A value for maximum Raw with DI was calculated by averaging maximum Raw values to challenges 2 and 4.

All data are presented as means ± SE. Statistical comparisons between the different conditions were made using ANOVA or t-test. For all statistical tests, P ≤ 0.05 was considered statistically significant.

**RESULTS**

The effect of a DI during bronchoconstriction on the increase in Raw in response to MCh is illustrated in Fig. 1 for a representative rabbit. The responses to challenges 1, 3, and 5 are under static conditions (no DI), and the responses to challenges 2 and 4 are with a DI immediately after the onset of bronchoconstriction. As illustrated in Fig. 1, the increase in Raw over baseline was consistently smaller when a DI was performed during bronchoconstriction. In both mature and immature rabbits, the peak increase in Raw was significantly smaller when a DI was performed after the onset of bronchoconstriction than under static conditions (Fig. 2). There were no significant differences in baseline resistances measured before MCh challenge with and without DI. We also observed no effect of either atropine or vagotomy on baseline Raw in the absence of ACh.

The inhibitory effect of a DI imposed early in the onset of the airway response to MCh did not result simply from a delay in achieving the peak increase in Raw. This is particularly evident at the higher dose of MCh for which the responses tended to be more prolonged. As shown in the examples shown in Fig. 3, even though the time course of the response to MCh is not delayed, the magnitude of the peak response is depressed by the DI.

The effect of four DIs before the MCh challenge on the airway response to MCh is illustrated in Fig. 4. Responses 1, 3, and 5 were obtained under static conditions (no DI), and responses 2 and 4 were obtained with four DIs performed immediately before MCh challenge. The increase of Raw above baseline was consistently smaller when DIs were performed before bronchoconstriction than under static conditions. In both mature and immature rabbits, the peak increase in Raw was significantly smaller when a DI was performed before the onset of bronchoconstriction than under static conditions (Fig. 5). There were no significant differences in baseline Raw measured before MCh challenge with and without DI.

The ability of DI to attenuate the airway response to MCh was expressed as the percent inhibition of the fold increase in Raw under static conditions (no DI). A DI performed during bronchoconstriction caused significantly greater inhibition of the response to MCh than DI performed before bronchoconstriction in both mature and immature animals (Fig. 6). There were no significant differences in the effects of DI on the responses to MCh in mature and immature animals.

**DISCUSSION**

We found that DIs before challenge with intravenous MCh or after the onset of bronchoconstriction significantly inhibited the increase in Raw in response to MCh. The inhibition of the airway response was significantly greater when the DI was performed immediately after MCh challenge than when it was performed before the delivery of MCh. There were no significant differences between mature and immature rabbits in the effects of DI on the airway response to MCh.

A number of mechanisms have been proposed to account for the effects of DI on airway responsiveness.
Stretches or length perturbations imposed on actively contracted airway smooth muscle in vitro inhibit subsequent force generation and shortening velocity during the contraction (4, 6, 9, 11, 24), suggesting that the effects of a DI on airway responsiveness in vivo during bronchoconstriction might be caused by the direct effects of stretch on the airway smooth muscle. It has been suggested that stretch of the muscle disrupts cross-bridge attachments and that the subsequent reattachment of cross bridges is impaired by the waning activation state of the muscle and the formation of latch bridges (5, 15, 25). Shinozuka et al. (25) suggested that the airway response to MCh was not depressed by the DI but merely delayed and that the effect of DI was to temporarily disrupt the normal bronchoconstrictor response. In our study, the duration of inflation was shorter than in the study of Shinozuka et al., whereas the inflation volume and the resulting peak airway pressure from the DI were greater. Under these conditions, it was evident in many of the rabbits that the time course of the response to a DI imposed after MCh challenge was unaltered and that the DI depressed the response to MCh (see Figs. 1, 3, and 4). In addition, an effect of stretch on cross-bridge cycling or muscle activation cannot account for our observation that volume maneuvers imposed before MCh challenge cause significant inhibition of the response to a subsequent MCh challenge. When we imposed a series of four DIs on the rabbits immediately before MCh challenge.

Fig. 3. Changes in Raw with time during challenge with a higher dose of 0.01 mg/kg MCh. Solid lines, airway response under static conditions (no DI); shadowed lines, response to MCh interrupted by a DI.

Fig. 4. Changes in Raw with time during MCh challenge in a representative rabbit. Responses to challenges 1, 3, and 5 (solid lines) were obtained under static conditions (no DI), and responses to challenges 2 and 4 (shadowed lines) were obtained after 4 DIs performed before the MCh challenge.

Fig. 5. In both mature and immature rabbits, the increase in Raw was significantly greater during MCh challenge under static conditions (no DI) than when the MCh challenge was performed after 4 DIs. Values are means ± SE. *P < 0.05.
The airway response to MCh was significantly inhibited but to a lesser degree than when the volume maneuver was imposed immediately after the MCh challenge. These results are unlikely to be explained by airway tone present before administration of the MCh challenge. We observed no effect of either vagotomy or atropine on baseline Raw in these rabbits. Other investigators have also reported that neither vagotomy nor atropine affects baseline Raw in rabbits (13, 26).

We have hypothesized that the effects of stretch on airway smooth muscle may result from a length-adaptive property of the airway smooth muscle in which the structural organization of the contractile apparatus is regulated by the cell in response to externally imposed changes in cell shape (7–10, 24). The smooth muscle cell may be able to optimize its contractility to changes in length or shape imposed by its external environment by acute adaptations in the organization of its cytoskeleton (8, 20). Stretch of the muscle may disrupt the organization of muscle structure, forcing it to adjust to a longer cell length. When the muscle is returned to a shorter length, readaptation of its structure to the shorter length may be slow, resulting in an immediate depression of contractility. This mechanism could account for the depression of the airway response that occurs when a DI is imposed either before or during MCh stimulation, because stretch of the muscle caused by the DI should disrupt the structural organization of the muscle under either condition. Differences in the magnitude of the effect of DI before vs. during bronchoconstriction may indicate that the activated muscle is less able to modulate its structure to adapt to an imposed change in cell length or that more than a single mechanism is involved. The disruption of cross-bridge attachments may also contribute to the effects of a DI when it is imposed after MCh challenge, resulting in a greater effect of DI under these conditions (4, 24).

Our observation that a DI imposed immediately before MCh challenge results in less inhibition of the airway response to MCh than a DI imposed after the onset of the airway response is consistent with previous observations of the relative effects of stretch on the contractility of airway smooth muscle strips in vitro (6, 17). In these studies, stretch of the muscle before contraction was less effective than stretch during contraction in inhibiting force development in response to ACh. However, our observations in rabbits contrast with the findings of both Malmberg et al. (16) and Scichilone et al. (21), in human subjects, who reported a greater inhibition of airway responsiveness when DI was imposed before the inhalation of MCh relative to a DI imposed subsequent to MCh inhalation. The differences in the relative effectiveness of DI before and after MCh in our study vs. the human studies might have resulted from a number of factors. In both of the studies on humans, airway responsiveness was assessed from measurements of forced expiratory volume in 1 s (FEV1), whereas, in our study, Raw was continuously monitored and airway narrowing was assessed in the absence of tidal breathing. In the human studies, the time course of the airway response to MCh and the timing of the DI relative to maximal airway narrowing may have differed from that in our studies in rabbits. In the human studies, it is unclear when maximal airway narrowing occurred relative to the measurement of FEV1. A DI followed inhalation of MCh, and FEV1 was measured ~3 min later. The FEV1 may not have been performed at the point of maximal airway response. In our study, a DI followed the intravenous MCh dose and the maximal airway response was measured <1 min after the DI (Fig. 1). Methodological differences between the protocols in our study and the studies in humans may account for the different results with respect to the relative effects of DI before and after MCh on airway responsiveness.

Asthmatic subjects exhibit greater airway narrowing and less bronchodilation in response to a deep breath compared with nonasthmatic subjects (1, 2, 19, 33). We previously observed that tidal ventilation inhibits the magnitude of the airway response to MCh in rabbits and that the degree of inhibition is proportional to tidal volume (23). We therefore hypothesized that the bronchodilating effect of a deep breath might be smaller in immature animals, which exhibit greater maximal airway narrowing than mature animals (22, 27–30). In the present study, DI caused inhibition of the airway response to MCh in both mature and immature rabbits. The difference in the effect of DI on immature rabbits and human asthmatic subjects suggests that the mechanism for airway hyperreactivity in immature rabbits may differ from that in asthmatic subjects. Our laboratory’s previous experiments suggest that the increased airway responsiveness observed in immature animals is related to the greater compliance of their airways and reduced interdependence between the airways and parenchymal tissue (29, 31). In contrast, airway inflammation may play an important role in mediating the hyperresponsiveness of asthmatic subjects. Factors associated with airway inflammation, such as edema or airway smooth muscle proliferation...
Institute Grants HL-48522 and HL-29289. Contributes to depression of the airway response. That reorganization of the contractile apparatus caused mechanisms. Our results are consistent with the possibility that reorganization of the contractile apparatus caused by stretch of the airway smooth muscle during DI contributes to depression of the airway response.

This work was supported by National Heart, Lung, and Blood Institute Grants HL-48522 and HL-29289.

REFERENCES