Methacholine causes reflex bronchoconstriction

ELIZABETH M. WAGNER AND DAVID B. JACOBY

Divisions of Pulmonary and Critical Care Medicine and of Physiology,
The Johns Hopkins University, Baltimore, Maryland 21224

Wagner, Elizabeth M., and David B. Jacoby. Methacholine causes reflex bronchoconstriction. J. Appl. Physiol. 86(1): 294–297, 1999.—To determine whether methacholine causes vagally mediated reflex constriction of airway smooth muscle, we administered methacholine to sheep either via the bronchial artery or via aerosol into the lower airways. We then measured the contraction of an isolated, in situ segment of trachealis smooth muscle and determined the effect of vagotomy on the trachealis response. Administering methacholine to the subcarinal arteries via the bronchial artery (0.5–10.0 µg/ml) caused dose-dependent bronchoconstriction and contraction of the tracheal segment. At the highest methacholine concentration delivered, trachealis smooth muscle tension increased an average of 186% over baseline. Aerosolized methacholine (5–7 breaths of 100 mg/ml) increased trachealis tension by 58% and airways resistance by 183%. As the bronchial circulation in the sheep does not supply the trachea, we postulated that the trachealis contraction was caused by a reflex response to methacholine in the lower airways. Bilateral vagotomy essentially eliminated the trachealis response and the airways resistance change after lower airways challenge (either via the bronchial artery or via aerosol) with methacholine. We conclude that 1) methacholine causes a substantial reflex contraction of airway smooth muscle and 2) the assumption may not be valid that a response to methacholine in humans or experimental animals represents solely the direct effect on smooth muscle.

METHODS

Experimental preparation. Approval for the described experimental protocols was obtained from the Johns Hopkins Animal Care and Use Committee. Anesthesia of healthy sheep of mixed breeds was induced with ketamine (1 g im) and maintained throughout the surgical and experimental periods with pentobarbital sodium (15 mg/kg loading dose, and 20 mg·kg⁻¹·h⁻¹). Sheep were paralysed with a constant infusion of succinylcholine (10 µg·kg⁻¹·min⁻¹), tracheostomized low in the neck, and mechanically ventilated via an 8-mm cuffed endotracheal tube with warmed, humidified air at a tidal volume (12 ml/kg) and rate (12–15 breaths/min) sufficient to ensure blood gases were within the normal range. Supplemental O₂ was provided, and, after thoracotomy, all sheep were placed on 5 cmH₂O positive end-expiratory pressure. Airway inflation pressure was measured at a side arm of the endotracheal tube. The vagus nerves were isolated bilaterally, and a segment of the trachea several centimeters rostral to the tracheostomy was isolated, as described by Brown and colleagues (2). Briefly, a horizontal incision was made between two cartilaginous rings and was extended to the edges of the trachealis muscle. A second similar incision was made three rings rostral to the first, and a vertical anterior midline incision was made to connect the two horizontal incisions. Thus a segment of trachea was created in which the trachealis could be sutured, via the attached cartilage, to a metal bar on one side and to a force transducer on the other side (Fig. 1). Contractions of the trachealis could then be recorded, via the force transducer, on a Grass recorder. Baseline tension was adjusted to ~30 g, as was done previously (2).

Catheters were inserted into the femoral vein for anesthitic infusion, into the femoral artery for systemic arterial pressure measurement, and into the other femoral artery to supply the pump used to perfuse the bronchial artery. A left lateral thoracotomy was performed between the fifth and
sixth ribs to allow direct access to the bronchoesophageal artery as it exits the aorta. The thoracic tracheal and esophageal branches of the bronchoesophageal artery were ligated. After intravenous infusion of the anticoagulant heparin (20,000 USP units), the bronchial artery was cannulated with an 18-gauge catheter inserted directly into the vessel. The catheter was secured in place, and the bronchial branch of the bronchoesophageal artery was perfused with autologous blood from the femoral artery at a flow controlled by a calibrated roller pump (Gilson) and set initially at a flow of 0.6 ml·min⁻¹·kg⁻¹, a value within the reported normal range for sheep (4). Bronchial artery pressure was measured at a side port of the inflow cannula. Measurements of hemodynamic variables were made with Gould transducers and recorded on a Grass recorder. To prevent the potential complicating interactions of the sympathetic nervous system during subsequent experimental interventions, guanethidine (5 mg/kg iv) was given. A 30-min stabilization period followed surgery.

Airway responses to methacholine. Methacholine was administered either by direct infusion into the bronchial perfusion circuit (0.5–10.0 µg/ml) or as an aerosol (5–7 breaths of 100 mg/ml; no. 646 Devilbiss) via the endotracheal tube. Lower airway responses were measured as an increase in airway inflation pressure. Tracheal responses were measured as an increase in tension of the isolated tracheal segment. After each methacholine challenge, complete recovery of tracheal tension to baseline was allowed before the next methacholine concentration was administered. During all airway challenges, the animals were ventilated with 100% O₂ to eliminate the potential confounding effects of hypoxemia (10). Methacholine challenge was performed before and after bilateral vagotomy.

In a separate set of experiments, increases in airways resistance after challenge with aerosolized methacholine were measured by using the technique of forced oscillation (8). After determining the response to methacholine aerosol (as above), the vagi were cut, and the protocol was repeated.

Data analysis. Changes in tracheal tension and airways resistance before and after vagotomy were assessed by using the Wilcoxon signed-ranks test. The one-tailed P values, if ≤0.05, were accepted as significant. All data are presented as means ± SE.

RESULTS

Bronchial blood flow for the entire group of nine sheep (29 ± 1 kg body weight) averaged 17 ± 1 ml/min and resulted in a bronchial artery perfusion pressure of
122 ± 11 mmHg. Average systemic arterial pressure in this group of animals was 91 ± 5 mmHg, and baseline peak airway inflation pressure averaged 17 ± 1 cmH₂O. Baseline tracheal tension was set to 36 ± 2 g.

In the first series of experiments (n = 4), intrabronchial artery infusion of methacholine caused dose-dependent bronchoconstriction that was reflected by an increase in airway inflation pressure and contraction of the trachealis muscle in the isolated tracheal segment. A comparison of trachealis and lower airways responsiveness in one representative animal is shown in Fig. 2, both before and after vagotomy, for two doses of methacholine. For the group of four sheep studied, airway pressure increased to a maximum of 42 ± 5 cmH₂O at the highest dose of methacholine given. The average dose-dependent increases in trachealis tension are presented in Fig. 3. The maximum methacholine concentration delivered to the subcarinal airways elicited, on average, an 88% increase in tracheal tension. Subsequent bilateral vagotomy decreased tracheal tension to 12 ± 4 g (68% reduction from baseline) but had no effect on overall airway pressure (20 ± 2 cmH₂O). Tracheal tension was reset close to the previous baseline level (32 ± 2 g) for the postvagotomy challenges. In Fig. 3, the average increase in tracheal tension for the group after vagotomy is compared with the prevagotomy responses. The change in tracheal tension observed with intrabronchial artery methacholine infusion was essentially eliminated after vagotomy. The small increases in tension seen at the two highest concentrations were due to the response in one animal.

Delivery of methacholine to the airways by aerosol (n = 4 sheep) resulted in an average 58% increase in tracheal segment tension. The increase in tension can be seen in Fig. 4. Bilateral vagotomy decreased this response almost completely, with an 8% response remaining (P = 0.034). In another group of animals (n = 4), the effect of aerosol methacholine on conducting airways resistance was determined. Baseline resistance before challenge was 2.4 ± 1.0 cmH₂O·l⁻¹·s. As can be seen in Fig. 5, methacholine challenge resulted in a 3.7 ± 1.1 cmH₂O·l⁻¹·s (183%) increase in resistance. Vagotomy resulted in a decline in baseline airways resistance in each animal (group average: 1.1 ± 0.2 cmH₂O·l⁻¹·s; P = 0.033). After vagotomy, the increase in resistance observed after methacholine challenge was 0.3 ± 0.1 cmH₂O·l⁻¹·s (P = 0.034 from prevagotomy).

**DISCUSSION**

The results of this study demonstrate that methacholine, whether delivered to the subcarinal airways via an intra-arterial route or aerosolized into the lower airways, causes a marked contraction of the isolated, in situ trachealis smooth muscle. Because this contraction was essentially eliminated by bilateral vagotomy, the present study suggests a reflex-mediated response and excludes the possibility that trachealis contraction was the result of recirculation of methacholine through the systemic circulation to the tracheal vessels of the animal.

![Graph showing changes in tracheal smooth muscle tension during intrabronchial artery challenge with 5 different MCh concentrations before (●) and after (○) bilateral vagotomy; n = 4 animals in each condition.](image1)

![Graph showing change in tracheal tension before (Pre-) and after (Post-) vagotomy after aerosol MCh challenge; n = 4 animals in each condition. *P = 0.034.](image2)

![Graph showing change in airway resistance before (Pre-) and after (Post-) vagotomy after aerosol challenge; n = 4 animals in each condition. *P = 0.034.](image3)
isolated segment. Furthermore, the lower airway response to aerosolized methacholine was also reduced by vagotomy, demonstrating that, in addition to a direct effect on lower airway smooth muscle, aerosolized methacholine-induced bronchoconstriction has a vagally mediated reflex component. This observation confirms our previous work in sheep, in which as much as 30% of the airways resistance increase after intrabronchial artery infusion of methacholine was eliminated after bilateral vagotomy (13).

The precise stimulus for the reflex-mediated increase in trachealis smooth muscle tension and enhanced lower airways constriction is not clear. Furthermore, it is not known whether methacholine delivered to the lower airways directly stimulates irritant receptors or whether smooth muscle constriction and airway narrowing stimulates stretch receptors, thereby causing an increase in afferent nerve activity. However, Dixon et al. (6) measured sensory nerve activity in dogs, and they found it to be substantially increased during challenge with a cholinergic agonist. Paradoxically, they found no reflex bronchoconstriction. Sorkness and colleagues (11) showed that intravenous challenge with methacholine and simultaneous vagal stimulation had a “supra-additive” effect on the airways response in rats. This was an unexpected observation, because bethanechol, a comparable nonselective muscarinic agonist, showed merely additive effects with vagal stimulation. The authors explained their results as demonstrating the capacity of methacholine to activate nicotinic cholinergic receptors on postganglionic parasympathetic neurons and on sensory afferent neurons in the airways. Tachykinins released by sensory fibers might facilitate enhanced bronchoconstriction. The extent to which sensory afferent nerves in the sheep contribute to airway control is not well defined.

It has long been recognized that airways hyperresponsiveness, frequently demonstrated as an increased bronchoconstriction in response to inhaled methacholine, is found in most patients with asthma. Despite this in vivo hyperresponsiveness, in airways taken from asthmatic subjects, in vitro contractile responses to cholinergic agonists have generally been found to be similar to those responses in tissues from normal subjects (1, 7, 14). Multiple explanations of this apparent paradox have been proposed, drawing on structural abnormalities of the airways (smaller baseline airway caliber, thicker airway walls, increased mucosal permeability), abnormal airway-parenchymal interdependence, and neural abnormalities. The potential role of abnormal vagally mediated reflex bronchoconstriction in causing airways hyperresponsiveness is frequently overlooked because of the assumption that methacholine causes bronchoconstriction exclusively via a direct effect on airway smooth muscle. Abnormalities of either the afferent or the efferent portions of the reflex arc may participate in airways hyperresponsiveness to methacholine.

In summary, we have shown that in sheep, methacholine causes a vagally mediated reflex contraction of smooth muscle in both the trachea and the conducting airways. Assumptions concerning the mechanisms of airway smooth muscle hyperresponsiveness that are based on methacholine working exclusively as a direct smooth muscle agonist may not, therefore, be valid.

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Address for reprint requests: E. M. Wagner, Division of Pulmonary and Critical Care Medicine, The Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Cir., Baltimore, MD 21224 (E-mail: wagnerem@welchlink.welch.jhu.edu).

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