Multiple mechanisms of reflex bronchospasm in guinea pigs

BRENDAN J. CANNING, SANDRA M. REYNOLDS, AND STUART B. MAZZONE
The Johns Hopkins Asthma and Allergy Center, Baltimore, Maryland 21224

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Canning, Brendan J., Sandra M. Reynolds, and Stuart B. Mazzone. Multiple mechanisms of reflex bronchospasm in guinea pigs. J Appl Physiol 91: 2642–2653, 2001.—The mechanisms of histamine- and bradykinin-induced reflex bronchospasm were determined in anesthetized guinea pigs. With intravenous administration, both autacoids evoked dose-dependent increases in tracheal cholinergic tone. Vagotomy or atropine prevented these tracheal reflexes. When delivered as an aerosol, bradykinin readily increased tracheal cholinergic tone, whereas histamine aerosols were much less effective at inducing tracheal reflexes. Also, unlike histamine, bradykinin could evoke profound increases in cholinergic tone without directly or indirectly (e.g., prostanoid dependent) inducing measurable airway smooth muscle contraction resulting in bronchospasm. Neither autacoid required de novo synthesis of prostanooids or nitric oxide to induce reflex tracheal contractions. Combined cyclooxygenase inhibition and tachykinin-receptor antagonism did, however, abolish all effects of bradykinin in the airways, whereas responses to histamine were unaffected by these pretreatments. The data indicate that histamine and bradykinin initiate reflex bronchospasm by differential activation of vagal afferent nerve subtypes. We speculate that selective activation of either airway C fibers or airway rapid adapting receptors can initiate reflex bronchospasm.

ATUCOIDS SUCH AS HISTAMINE and bradykinin are thought to initiate airway defensive reflexes such as cough and bronchospasm secondary to activation of vagal afferent nerves innervating the larynx, trachea, bronchi, and lungs (7, 9–11). Airway afferent nerves have been subclassified on the basis of physicochemical sensitivity, location within the airways and lungs, adaptation to sustained lung inflations, neurochemistry, origin, and action potential conduction velocities (10, 23, 35, 47). Although considerable heterogeneity exists, these afferents have been subclassified into three broad groups: unmyelinated, nociceptive type C fibers; rapidly adapting (or “irritant”) mechanoreceptors (RARs); and slowly adapting stretch receptors (SARs). It has not been clearly established which afferent nerves mediate the reflexes initiated by histamine and bradykinin (25, 42, 44, 51).

Stimuli that activate airway RARs or nociceptive type C fibers produce reflex-mediated cholinergic nerve-dependent bronchospasm in most animals and in humans (9, 15, 25, 40, 43). This has led to the hypothesis that both RARs and airway C fibers regulate lung parasympathetic nerve activity. Many stimuli thought to be selective for either airway C fibers or RARs are, however, nonselective stimulants. Autacoids such as histamine and serotonin, for example, relatively selective stimulants of RARs (4, 11, 29), can also activate at least some airway C fibers (9, 24). Conversely, capsaicin, putatively selective for stimulating unmyelinated nociceptors throughout the peripheral nervous system (46), activates both myelinated and unmyelinated afferents innervating the guinea pig airways in vitro and might, through direct or indirect mechanisms, activate RARs in vivo (3, 30, 36).

Further complication comes from the ability of these stimuli to induce formation of prostanooids and/or nitric oxide, which might in turn activate multiple airway afferent nerve subtypes (9, 19, 31, 35, 45). Histamine challenge or challenge with other mediators of the acute allergic response might even induce bradykinin formation, which can activate airway C fibers (5).

A potentially useful distinction between unmyelinated C fibers and RARs innervating the airways is their differential expression of the tachykinins substance P and neurokinin A. In healthy guinea pigs, only unmyelinated airway C fibers express the tachykinins (23, 35). It may be possible, therefore, to utilize tachykinin-receptor antagonists as a way to distinguish the actions of airway C fibers from the actions of RARs. Also taking advantage of the highly selective actions of histamine and bradykinin on RARs and C fibers in guinea pigs (3, 20, 36, 37) and measurements of cholinergic tone in the trachea (TT) in situ, we utilized this study to define the mechanisms by which reflex bronchospasm is initiated. Furthermore, the hypothesis that activation of multiple airway afferent nerve subtypes initiates increases in airway parasympathetic nerve activity was addressed.

METHODS

Animal preparation. All experiments described below were approved by the Johns Hopkins Medical Institutions Animal.
Care and Use Committee. Male Hartley guinea pigs (300–400 g, Hilltop, Scottsdale, PA) were anesthetized with urethane (1 g/kg ip) and positioned ventral side up on a heated pad. The caudalmost portion of the extrathoracic trachea was cannulated, and the animals were mechanically ventilated (60 breaths/min, 6 ml/kg, 2–3 cmH2O of positive end-expiratory pressure) after paralysis (2 mg/kg succinylcholine chloride given subcutaneously).

TT was measured in situ as described previously (21). Stainless steel hooks were passed between two cartilage rings (rings 6 and 7 caudal to the larynx) on either side of the trachea, rostral to the tracheal cannula. One hook was sutured to a fixed bar, and the other hook was sutured to an isometric force transducer (model FT03C, Grass Instruments, Quincy, MA). Optimal baseline tension was set (1.5–2 g) and maintained throughout the equilibration period. The lumen of the tracheal segment studied was perfused with warmed (37°C), oxygenated Krebs buffer delivered through a small slit made in the ventral trachea, caudal to the hooks. The buffer was removed from the rostralmost end of the trachea by negative suction.

In all experiments described in this study, the Krebs bicarbonate buffer perfusing the tracheal lumen [composition (in mM): 118 NaCl, 5.4 KCl, 1 NaH2PO4, 1.2 MgSO4, 1.9 CaCl2, 25 NaHCO3, and 11.1 dextrose] contained 3 μM indomethacin, 2 μM propranolol, and 1 μM phentolamine. These drugs were used to block the local effects of prostaglandins and to block any effects of circulating and neurally released catecholamines on the tracheal segment studied. Unless otherwise stated, all animals were also pretreated with intravenous propranolol (1 mg/kg).

Tracheal blood flow is left undisturbed in this preparation. It is possible, therefore, that exogenously administered histamine or bradykinin could reach the trachea via the vasculature and have direct effects on tracheal smooth muscle tone. To prevent direct tracheal effects of these autacoids, the histamine H1-receptor antagonist pyrilamine (1 μM) and the bradykinin B2-receptor antagonist FR-173657 (0.3 μM) were added selectively to the tracheal perfusate. The adequacy of these pretreatments and the reflex nature of the tracheal responses evoked were confirmed by showing that bradykinin- and histamine-induced alterations in TT were completely abolished and/or reversed by atropine or vagotomy (see results).

The abdominal aorta was cannulated to monitor arterial blood pressure (ABP) and for intra-arterial drug injections. For the latter procedure, the cannula was advanced into the thoracic aorta to minimize the delivery of the agents to the abdominal viscera. For intravenous delivery of drugs, the abdominal vena cava was cannulated. Furthermore, in some experiments, a cannula fashioned of stainless steel was positioned in the right lateral cerebral ventricle (from bregma, 1.8 mm posterior, 2 mm lateral, 4.8 mm ventral from the top of the skull) to allow intracerebroventricular (icv) administration of drugs. Correct placement of the cannula was confirmed postmortem by using Evans blue dye injections. ABP, pulmonary insufflation pressure (PIP), and TT were recorded on a Grass polygraph (model 78E).

At the conclusion of each experiment, animals were killed by inhalation of 100% CO2, delivered through the inspiratory port of the ventilator.

Intravenous administration of histamine and bradykinin. When stable recordings of TT, PIP, and ABP were established, concentration-response curves to either histamine (0.5–20 μg/kg iv) or bradykinin (0.05–2 nmol/kg iv) were constructed. Subsequent doses of the autacoids were administered when the effects of the previous dose had returned to baseline. In some experiments, the autacoids were continuously infused by using a syringe pump. If necessary, lung hyperinflations (" sighs"; 2–3 tidal volumes) were performed to reverse any residual obstruction evoked by bolus administration of the agonists. The effects of bradykinin and histamine on PIP were expressed as a percent increase over baseline. The effects of the autacoids on TT were expressed as a percent increase in TT or as a percentage of the maximum contraction evoked by 300 mM BaCl2. Baseline TT was quantified by dividing the relaxation (in g) evoked by adding the muscarinic-receptor antagonist atropine (1 μM) to the tracheal perfusate by the subsequently evoked maximum contraction induced by BaCl2 (see Ref. 22).

We quantified the effects of the following treatments and interventions on bradykinin- and histamine-induced alterations of TT and PIP: 1) atropine (1 mg/kg iv or 1 μM in the tracheal perfusate); 2) the ganglionic blocker trimethaphan (5 mg/kg iv); 3) propranolol (1 mg/kg iv); 4) either the histamine H1-receptor antagonist pyrilamine (1 mg/kg iv) or the bradykinin B2-receptor antagonist HOE-140 (1 mg/kg iv); 5) atropine and histamine (tracheal perfusate); and 6) the neuropeptide Y-receptor antagonists (SR-140333, SR-48968, and SB-223412; 1 mg/kg each iv); 6) iv infusion (3.3 nmol/min for up to 30 min, beginning 10 min before and continuing during histamine or bradykinin challenge) of the nonselective neurokinin receptor antagonist ZD-6021 (27, 40) [previous studies have shown that the vehicle for ZD-6021 was without effect on reflexes initiated by histamine, bradykinin, or capsaicin and was without effect on baseline cholinergic tone (28)]; 7) cyclooxygenase inhibition with meclofenamic acid (3 mg/kg iv); 8) combined cyclooxygenase and nitric oxide synthase inhibition with meclofenamic acid and Nω-Nitro-l-arginine (11NNA) (3 and 5 mg/kg iv, respectively); 9) combined cyclooxygenase inhibition and tachykinin-receptor antagonism (treatments 5 and 7 above, respectively); 10) bilateral vagotomy (extrathoracic, subbronchial or just rostral to the mainstem bronchi); and 11) increasing positive end-expiratory pressure. These interventions were studied in either paired or nonpaired fashion. When necessary, vehicle control experiments were carried out in parallel.

In some experiments, the ability of histamine and bradykinin to evoke reflex tracheal relaxations through activation of airway noncholinergic-parasympathetic nerves was studied. Atropine was added to the tracheal perfusate to reverse baseline TT, and the tracheal is subsequently contracted (~50% of maximum contraction) with 1 μM PGF2α. When the contraction to PGF2α stabilized, either histamine (10 μg/kg) or bradykinin (1 nmol/kg) was administered intravenously. Relaxant responses evoked were quantified as a percent reversal of the PGF2α-induced contraction. The effects of ganglionic blockade with trimethaphan (5 mg/kg iv) on these relaxations were studied in a nonpaired experimental design.

Effects of nebulized bradykinin and histamine on PIP and TT. The effects of histamine and bradykinin delivered as aerosols were studied in a nonpaired experimental design. After the establishment of a stable baseline of TT, ABP, and PIP, the lower airways were challenged with either bradykinin (1 mg/ml) or histamine (1 μg–10 mg/ml) delivered using a DeVilbiss nebulizer (model 25; average particle size: 3 μm) connected in series to the ventilator and tracheal cannula. Aerosol challenges continued for up to 10 min and were terminated when responses had stabilized or when increases in PIP exceeded 150% of baseline.

Statistics. All data are presented as means ± SE. ED50 values were determined by visual inspection of individual graphed dose-response curves with responses normalized to the maximum effect evoked in each experiment. Differences between groups were assessed using ANOVA for
Macintosh (Berkeley, CA). Values of \( P < 0.05 \) were considered significant. When significant variation between groups was detected, treatment group means were compared by using Scheffé's \( F \) test for unplanned comparisons.

Occasionally (<10% of experiments), animals exhibited little (<10% of the maximum contraction) or no baseline TT or baseline TT that approximated the maximum TT (>50% of the maximum contraction) attainable in this preparation (see Ref. 22). Such preparations rarely exhibited any reflex effects in response to any stimulus and were thus excluded from subsequent statistical analyses.

**Drugs.** Atropine sulfate, BaCl\(_2\), heparin sulfate, indomethacin, histamine, urethane (ethyl carbamate), succinylcholine chloride, \( L \)-NNA, meclofenamic acid, bicuculline, pyrilamine, pentolamine hydrochloride, and \( DL \)-propranolol hydrochloride were purchased from Sigma Chemical (St. Louis, MO). PGF\(_{2\alpha}\) was purchased from Biomol (Plymouth, PA). Tri méthaphan camyslate was acquired from Roche Laboratories (Nutley, NJ). Leukotriene \( D_4 \) (LTD\(_4\)), SR-140333, and SB-223412 were provided by Glaxo SmithKline (King of Prussia, PA). SR-48968 was provided by AstraZeneca (Wilmington, DE). HOE-140 was provided by Aventis (Frankfurt, Germany). FR-173657 (1 mM) and bicuculline (10 nmol/kg) were dissolved in DMSO. Drugs administered intravenously, subcutaneously, or as an aerosol (1–50 mg/ml) were dissolved in saline with the following exceptions: SR-48968 and SR-140333 were dissolved in DMSO (10 mg/ml) and diluted (1 mg/ml) in saline, SR-140333 was dissolved in DMSO (10 mg/ml) and diluted (1 mg/ml) with acid in saline, L-NNA was dissolved in 0.1 N acid (30 mg/ml) and diluted (1 mg/ml) in saline, and LTD\(_4\) was dissolved in methanol (0.1 mM) and diluted (1 \( \mu g/ml \) in saline.

**RESULTS**

**Reflex bronchospasm evoked by histamine.** Intravenous administration of histamine evoked dose-dependent increases in PIP and TT (Fig. 1A). The kinetics of the effects of low doses of histamine (<5 \( \mu g/kg \) on TT mimicked the kinetics of the changes in PIP evoked by the autacoid. This mimicry was true only for the dynamic component of the response to histamine when continuously infused, histamine (10 \( \mu g \cdot kg^{-1} \cdot min^{-1} \)) evoked a sustained increase in PIP (89 ± 16% increase; Fig. 2B), but TT rose and remained elevated only during the increasing phase of the pulmonary response (Fig. 2A).

Unlike intravenously administered histamine, nebulized histamine (0.01–10 mg/ml) was less effective at evoking increases in TT (−7.4 ± 22.6% decrease in TT) despite inducing marked bronchospasm in all animals (60 ± 14% increase in PIP; \( n = 7 \)). In four of seven preparations, nebulized histamine had no effect or evoked only decreases in TT. In the remaining three preparations, nebulized histamine induced modest increases in TT that were either followed or preceded by decreases in TT. Nebulized saline was without effect on TT or PIP (\( n = 5 \)).

All reflex tracheal effects evoked by histamine were completely abolished by vagotomy or pretreatment with atropine, thereby confirming their reflex and cholinergic properties (Fig. 3, A and B; Table 1). Neither atropine nor vagotomy significantly altered the measured effects of histamine on PIP. The histamine H\(_1\)-receptor antagonist pyrilamine (1 mg/kg iv) abolished all effects of the autacoid (≤20 \( \mu g/kg \) iv) on the airways (\( n = 3 \); see Table 1).

**Reflex bronchospasm evoked by bradykinin.** Intravenous administration of bradykinin evoked dose-dependent increases in PIP and TT (Fig. 3, C and D). The effects of bradykinin on cholinergic tone were highly reproducible and more pronounced than the effects of histamine on the trachea. The kinetics of the response to bradykinin in the trachea also differed substantially from the response to histamine, increasing immediately but gradually on administration of the peptide and slowly returning to baseline over the ensuing 2–5 min. When bradykinin (1 nmol·kg\(^{-1}\)·min\(^{-1}\) iv) was continuously infused, TT rose and remained elevated for the duration of the infusion (\( n = 5 \); Fig. 2A). By contrast, the effects of bradykinin on PIP were less pronounced and often not as dose dependent relative to the effects of histamine (Figs. 2B and 3). Occasionally, low doses of bradykinin evoked marked changes in TT in the absence of measurable alterations in PIP. In some preparations, the effects of bradykinin on PIP seemed to desensitize, evoking only modest increases in PIP at high doses despite still evoking profound increases in TT and decreases in ABP. Atropine or vagotomy abolished the effects of bradykinin on TT (Fig. 3C, Table 2). By contrast, atropine or vagotomy only inhibited the effects of the peptide on PIP (Fig. 3D, Table 2). The bradykinin B\(_2\)-receptor antagonist HOE-140 (1 mg/kg iv) abolished the effects of all doses of the inflammatory peptide studied (≤2 nmol/kg iv; \( n = 3 \); see Table 2).

Unlike histamine, nebulized bradykinin readily increased TT (104 ± 7%; \( n = 8 \)), an effect that was prevented by vagotomy (\( n = 3 \)) or reversed by atropine (\( n = 8 \)) (Fig. 1).

**Role of alterations in pulmonary mechanics or PIP changes on reflex tracheal contractions evoked by histamine and bradykinin.** Severing the vagi caudal to the pulmonary branches of the vagus nerves had no effect on reflexes evoked by 5 \( \mu g/kg \) histamine or 1 nmol/kg bradykinin administered intravenously (37 ± 1 and 47 ± 10% increases in TT, respectively; \( n = 5 \)). In these same preparations, arterial administration of the identical doses of histamine or bradykinin had minimal effects on TT (4 ± 4 and 17 ± 3% increase, respectively; \( P < 0.05 \)) and no effect on PIP. This suggests that the histamine- and bradykinin-induced tracheal reflexes were mediated primarily by activation of airway afferent nerves. Consistent with this hypothesis, severing the nerves rostral to the pulmonary branches of the vagi (but caudal to the recurrent laryngeal nerves, which carry the tracheal preganglionic fibers) abolished histamine- and bradykinin-induced tracheal reflexes (\( n = 2–3 \)). These nerve cuts did not inhibit
bradykinin- or histamine-induced increases in PIP [125% (n = 2) and 145 ± 27% (n = 3), respectively].

The effects of both bradykinin and histamine on TT and PIP were closely correlated over a range of doses of both autacoids (histamine: \( r = 0.76 \); bradykinin: \( r = 0.64 \)). The slope (m) of this correlation was much steeper for bradykinin (histamine: m = 0.08; bradykinin: m = 0.5), indicating that the inflammatory peptide is more effective at evoking reflex effects than at producing airway obstruction through direct effects on the airways. These associations could merely indicate that the effects of the autacoids on airway smooth muscle and on airway afferent nerves are dependent on activation of the same receptor subtype (histamine H\(_1\) and bradykinin B\(_2\) receptors, respectively). Alternatively, the data might suggest that the peripheral, direct effects of these autacoids on pulmonary mechanics, particularly histamine, might contribute to their ability to evoke reflexes. In an attempt to distinguish between these two hypotheses, the effects of limiting smooth muscle functional antagonism produced by circulating and locally released catecholamines on the ability of histamine and bradykinin to evoke reflex tracheal contractions were assessed. Propranolol pretreatment markedly potentiated the effects of histamine on both PIP and TT while having little, if any,
H1-receptor antagonist pyrilamine (1 μmol/kg iv) and the bradykinin B2-receptor antagonist FR-173657 (0.3 μmol/kg iv) on TT (A) and PIP (B) in guinea pigs in situ. Values are means ± SE. Direct effects of bradykinin and histamine on the trachea were prevented in all experiments by adding the histamine H1-receptor antagonist pyrilamine (1 μM) and the bradykinin B2-receptor antagonist FR-173657 (0.3 μM) to the tracheal perfusate. Peak effects of the autacoids on TT in these experiments did not differ (P > 0.1). Effects of histamine on PIP were substantially greater than those evoked by bradykinin (n = 5; P < 0.01). Note that continuously infused bradykinin evoked a sustained increase in PIP and TT, whereas histamine evoked increases in TT only during the dynamic phase of the sustained increase in PIP.

Fig. 2. Effect of continuously infused (beginning at arrows) histamine (10 μg·kg⁻¹·min⁻¹ iv; ○) and bradykinin (1 nmol·kg⁻¹·min⁻¹ iv; ●) on TT (A) and PIP (B) in guinea pigs in situ. Values are means ± SE. Direct effects of bradykinin and histamine on the trachea were prevented in all experiments by adding the histamine H1-receptor antagonist pyrilamine (1 μM) and the bradykinin B2-receptor antagonist FR-173657 (0.3 μM) to the tracheal perfusate. Peak effects of the autacoids on TT in these experiments did not differ (P > 0.1). Effects of histamine on PIP were substantially greater than those evoked by bradykinin (n = 5; P < 0.01). Note that continuously infused bradykinin evoked a sustained increase in PIP and TT, whereas histamine evoked increases in TT only during the dynamic phase of the sustained increase in PIP.

The effects of bradykinin approached the maximum attainable nerve-mediated increases in TT at concentrations that evoked only marginal increases in PIP. This was most evident when bradykinin was delivered as an aerosol (Fig. 1) or when bradykinin was administered after cyclooxygenase inhibition with meclofenamic acid (Table 2). The effects of histamine on TT reached an asymptote well below the maximum attainable increases in airway parasympathetic cholinergic nerve effects. This asymptote occurred in the histamine concentration-response curve for TT despite the ability of the autacoids to evoke further increases in PIP (Fig. 3, A and B; Table 1). Perhaps a compensatory inhibitory reflex might blunt the effects of autacoids on reflex tracheal contractions. It is unlikely that histamine is more effective than bradykinin at activating airway noncholinergic parasympathetic relaxant nerves: both histamine and bradykinin evoked reflex tracheal relaxations with intravenous administration (Table 3). It is possible, however, that histamine, through its direct capacity to increase PIP, preferentially increases SAR activity (which will inhibit TT; see Ref. 22) during fixed-volume ventilation. Indeed, decreasing positive end-expiratory pressure decreased baseline tone and blunted the ability of histamine to evoke reflex tracheal contractions (Fig. 5).

Role of vascular effects in reflex bronchospasm evoked by histamine and bradykinin. Histamine and bradykinin can evoke airway smooth muscle contraction but also relaxation of pulmonary vascular smooth muscle (2, 14, 31). The effects of histamine and bradykinin on the vasculature are mediated indirectly through formation of prostanoids and nitric oxide, both of which are thought to modulate and/or activate airway afferent nerves. The role of prostanoids and nitric oxide in histamine- and bradykinin-induced responses were quantified by blocking cyclooxygenase and nitric oxide synthase with meclofenamic acid and L-NNA, respectively. Histamine (2 μg/kg iv) and bradykinin (0.5 nmol/kg iv) still evoked reflex tracheal contractions under these conditions (11 ± 3 and 6 ± 1% of the maximum contraction, respectively), indicating that neither nitric oxide nor prostanoids are necessary for bradykinin- or histamine-induced reflex bronchospasm. It is also unlikely that vasodilatation plays a role in mediating these reflexes. Further evidence for this latter assertion comes from studying the effects of the bronchoconstrictor and pulmonary vasconstrictor LTD4. In an animal pretreated intravenously with meclofenamic acid and L-NNA and intratracheally with the cysteiny1 leukotrine-receptor antagonist ICI-198615 (3 μM), LTD4 (1.5 nmol/kg iv) evoked a 52% increase in TT (and a 20% increase in PIP), an effect that was completely abolished by atropine.

Meclofenamic acid administered without L-NNA did not modulate histamine-mediated effects on either PIP or TT (Figs. 6B, 7A, and 7B; Table 1). The cyclooxygenase inhibitor was not completely ineffective at modulating responses evoked by the autacoids, however, as it essentially abolished the effects of bradykinin on PIP (Figs. 6A and 7D; Table 2). Nevertheless, bradykinin still readily evoked reflex tracheal contractions in the presence of meclofenamic acid (Figs. 6A and 7C, Table 2).

Consistent with previous studies (22), ABP appeared to have little, if any, influence over TT. Baseline TT was not correlated (r = 0.12) with mean ABP (MABP; which averaged 42 ± 3 mmHg in 12 representative control experiments). Similarly, the effects of histamine (r = 0.28) and bradykinin (r = 0.35) on MABP were also not correlated with their effects on TT. Indeed, whereas bradykinin consistently and dose-dependently decreased MABP, histamine had inconsistent effects on MABP.

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Role of tachykinins and capsaicin-sensitive nerves in reflex bronchospasm evoked by histamine and bradykinin. The role of tachykinins in histamine- and bradykinin-mediated airway effects was first assessed by using tachykinin receptor-selective antagonists [neurokinin-1 (SR-140333), neurokinin-2 (SR-48968), and neurokinin-3 (SB-223412) receptors, respectively]. The tachykinin-receptor antagonists (administered intravenously) had no inhibitory effects on baseline TT or histamine-mediated effects in the airways (Figs. 6 C, 7 A, and 7 B; Table 1). When administered alone, the antagonists were also without substantial effect on bradykinin-induced tracheal reflexes (Table 2). However, when the prostanoid-dependent bronchospasm mediated by bradykinin was blocked with meclofenamic acid, the neurokinin-receptor antagonists completely abolished the tracheal and pulmonary effects of bradykinin (Figs. 6 C, 7 C, and 7 D; Table 2). In a second series of experiments, icv infusion of the nonselective neurokinin-receptor antagonist ZD-6021 (in meclofenamic acid-pretreated guinea pigs) abolished reflex tracheal contractions evoked by intravenous bradykinin (Table 2) but not those evoked by histamine (Table 1).

Effects of interventions on baseline TT. Overall, baseline TT averaged 28.6 ± 1.7% of the maximum contraction evoked by BaCl₂ in the experiments summarized above (n = 55). Subbronchial vagotomy, propranolol

Table 1. Pharmacology of intravenous histamine-mediated airway effects in guinea pigs

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<tr>
<th></th>
<th>n</th>
<th>Maximum increasea</th>
<th>ED50, μg/kg</th>
<th>Maximum increaseb</th>
<th>ED50, μg/kg</th>
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<td>Histamine</td>
<td>10</td>
<td>195 ± 17e</td>
<td>8.1 ± 1.3e</td>
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<td>+1 mg/kg atropine</td>
<td>6</td>
<td>155 ± 42</td>
<td>7.7 ± 1.5</td>
<td>0.4 ± 0.4d</td>
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<td>+1 mg/kg pyrilamine</td>
<td>3</td>
<td>0 ± 0d</td>
<td>&gt;20.0d</td>
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<tr>
<td>+3 mg/kg meclofenamic acid</td>
<td>5</td>
<td>235 ± 28</td>
<td>6.3 ± 1.8</td>
<td>29.5 ± 3.8</td>
<td>3.1 ± 0.9</td>
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<tr>
<td>+3 mg/kg meclofenamic acid and 1 mg/kg each SR-140333, SR-48968, and SB-223412</td>
<td>5</td>
<td>182 ± 32</td>
<td>7.4 ± 1.3</td>
<td>24.7 ± 3.8</td>
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<tr>
<td>+3 mg/kg meclofenamic acid (iv) and 3.3 nmol/min (icv) ZD-6021</td>
<td>5</td>
<td>185 ± 8</td>
<td>3.1 ± 0.4d</td>
<td>27.9 ± 4.9</td>
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Values are means ± SE. ED50, dose of histamine required to produce 50% of the maximum response; icv, intracerebroventricular.

Fig. 3. Summary of the effects of histamine (A and B) and bradykinin (C and D) on TT (A and C) and PIP (B and D) in guinea pigs in situ. Direct effects of bradykinin and histamine on the trachea were prevented in all experiments by adding the histamine H₁-receptor antagonist pyrilamine (1 μM) and the bradykinin B₂-receptor antagonist FR-173657 (0.3 μM) to the tracheal perfusate. Effects of histamine and bradykinin were studied in the absence (●) and presence (○) of atropine (1 mg/kg iv). Each point is the mean ± SE of 5–10 experiments. See text for details of the experimental design. Also see Tables 1 and 2. Effects of atropine on baseline and reflex-mediated alterations in TT and PIP were mimicked by vagotomy (data not shown).
(1 mg/kg iv), meclofenamic acid (3 mg/kg iv) and tachykinin-receptor antagonists [SR-140333, SR-48968, SB-223412 (1 mg/kg each iv)] or ZD-6021 (3.3 nmol/min icv) failed to evoke sustained alterations in baseline TT (n = 5–55). By contrast, increasing positive end-expiratory pressure (10 cmH2O) decreased TT (220 ± 61%), whereas 5 mg/kg L-NNA combined with meclofenamic acid increased baseline TT (80 ± 50%; n = 3–4). As expected (22), bilateral vagotomy (either cervical or just caudal to the recurrent laryngeal nerves) effectively abolished baseline TT (90–100% reduction).

DISCUSSION

This study provides physiological and pharmacological evidence that bradykinin and histamine evoke reflex bronchospasm in guinea pigs by activating different airway afferent nerve subtypes. Furthermore, the data presented indicate that histamine evokes its effects on airway afferent nerves through indirect mechanisms, perhaps secondary to smooth muscle contraction and bronchospasm. These observations have important implications on how airway reflexes are precipitated and provide evidence for potential interactions between the actions of airway afferent nerve subtypes on autonomic tone, cough, and respiratory rhythm.

Mechanisms of reflex bronchospasm evoked by histamine and bradykinin: evidence for distinct afferent nerve subtypes initiating reflex bronchospasm. Purely electrophysiological approaches to addressing hypotheses that specific afferent nerve subtypes mediate clearly defined airway defensive reflexes are inadequate. Even subtle changes, either increases or decreases, in the activity of just a few afferent nerves within a specific subtype make it possible that the

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<th>Bradykinin</th>
<th>Pulmonary Insufflation Pressure</th>
<th>Tracheal Tone</th>
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<tr>
<td></td>
<td>Maximum increase</td>
<td>ED50, nmol/kg</td>
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</tr>
<tr>
<td>+3 mg/kg meclofenamic acid and 1 mg/kg each SR-140333, SR-48968, SB-223412</td>
<td>6</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>+3 mg/kg meclofenamic acid (iv) and 3.3 nmol/min icv ZD-6021</td>
<td>5</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

Values are means ± SE. ED50, dose of bradykinin required to produce 50% of the maximum response. *Significantly different from corresponding histamine value, P < 0.001 (Table 1).
Table 3. Reflex-mediated relaxations of the guinea pig trachea in situ evoked by intravenous administration of histamine or bradykinin

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Insufflation Pressure, %increase</th>
<th>Tracheal Tone (effect on PGF₂α contraction), %reversal</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μg/kg Histamine</td>
<td>6</td>
<td>170 ± 2</td>
</tr>
<tr>
<td>+5 mg/kg trimethaphan</td>
<td>3</td>
<td>114 ± 55</td>
</tr>
<tr>
<td>1 nmol/kg Bradykinin</td>
<td>5</td>
<td>177 ± 42</td>
</tr>
<tr>
<td>+5 mg/kg trimethaphan</td>
<td>3</td>
<td>94 ± 19</td>
</tr>
</tbody>
</table>

Values are means ± SE. Preparations were pretreated with atropine (1 μM intratracheally), and trachealis was precontracted by adding 1 μM PGE₂, to tracheal perfusate. *Significantly less reversal of the PGF₂α contraction in the presence of trimethaphan, P = 0.05.

afferent nerves affected contribute to the reflexes studied. Similarly, a limited pharmacological approach is also inadequate for addressing hypotheses that specific afferent nerve subtypes mediate specific airway reflexes. Thus it is clear that, whereas selectivity is possible in a pharmacological analysis, specificity is rarely achieved. It may also be inappropriate to draw meaningful conclusions from negative results. The inability of stimuli thought to be selective for activating specific afferent nerve subtypes to evoke specific reflexes does not necessarily rule out the role of those afferent nerves in the reflexes studied. Clearly then, it is necessary to rely heavily on the well-established actions of specific stimuli on the activity of well-defined afferent nerve subtypes when addressing hypotheses about the role of specific afferent nerves in mediating clearly defined reflexes. It is also important to have a sensitive and accurate measure of the reflex studied.

Three lines of experimental evidence indicate that histamine and bradykinin initiate reflex bronchospasm by activating distinct airway afferent nerve subtypes and do so by distinct mechanisms. First, bradykinin, unlike histamine, is a direct stimulant of tachykinin-containing vagal afferent nerves in vitro, whereas it has no direct effects on RARs innervating the trachea, larynx, and bronchi and has only subtle, direct effects on airway smooth muscle (9, 14, 21, 35, 38). Tachykinin-receptor antagonists were without effect on baseline TT or histamine-induced increases in TT but abolished reflexes initiated by bradykinin (when cyclooxygenase was also inhibited). Second, in vivo, activation of airway afferent nerves by histamine can be prevented by counteracting the coincident bronchospasm produced by the autacoid (4). The β-adrenoceptor antagonist propranolol potentiated histamine-induced bronchospasm and reflex tracheal contractions, whereas it had no effect on bradykinin-induced increases in TT. This effect was attributable to the ability of propranolol to counteract smooth muscle functional antagonism by endogenous catecholamines released from nerves or from the adrenal glands (12). Finally, bradykinin consistently evoked reflex bronchospasm when delivered via aerosol, whereas histamine failed to readily evoke reflex bronchospasm when delivered as an aerosol, despite evoking marked increases in PIP.

Histamine may initiate reflexes in the airways through indirect effects on airway afferent nerves that are physiologically similar to airway RARs. This assertion is supported by several lines of experimental evidence. 1) In vivo electrophysiological studies document the ability of histamine or any stimulus that reduces dynamic pulmonary compliance to activate pulmonary RARs (4, 33, 40). 2) Decreasing pulmonary compliance by increasing pleural pressure (while maintaining tidal volume and respiratory rate) mimics exactly the effects of histamine on TT (S. B. Mazzone, unpublished observations). 3) Histamine fails to markedly alter airway C-fiber activity in vivo unless it evokes profound bronchospasm, and it fails to directly activate tracheal or bronchial RARs or C fibers in vitro (24, 36). 4) Reflex alterations in TT evoked by histamine rapidly adapt to a sustained intravenous challenge with the autacoid (Fig. 2). 5) Tachykinin-receptor antagonists inhibit or abolish reflexes thought to be mediated by airway C fibers (1, 7, 26, 27). Tachykinin-receptor antagonists were without inhibitory effects on reflexes evoked by histamine in the present study. 6) Activation of SARs evokes bronchodilatation, not bronchospasm (22, 37).

Reflexes evoked by bradykinin are due in large part to the direct effects of the peptide on tachykinin-containing C fibers innervating the airways and lungs.
Bradykinin can also activate Aδ fibers innervating the airways, which are responsive to capsaicin but do not synthesize tachykinins under normal, noninflamed conditions (21, 35). It is possible that a component of the reflex effects produced by bradykinin in guinea pigs depends on activation of the nociceptive-type Aδ fibers. Given that the tachykinin-receptor antagonists completely abolish the effects of bradykinin, however, the data indicate that activation of Aδ fibers by bradykinin is not sufficient for inducing reflex bronchospasm. Furthermore, because meclofenamic acid abolished all measurable, nonneural effects of bradykinin, and tachykinin-receptor antagonists act in the central nervous system (CNS) to prevent other C-fiber-mediated effects of bradykinin, we conclude that the reflex bronchospastic actions of bradykinin depend on activation of Aδ fibers by bradykinin.

Fig. 6. Representative traces showing the effects of the cyclooxygenase inhibitor meclofenamic acid (3 mg/kg iv) and the tachykinin-receptor antagonists SR-140333, SR-48968, and SB-223412 (1 mg/kg iv each) on bradykinin- and histamine-induced increases in PIP and TT in guinea pigs in situ. A and B: meclofenamic acid effectively abolished bradykinin-induced increases in PIP while having little, if any, effect on the peak increases in TT evoked by either histamine or bradykinin or on the effects of histamine on PIP. C: co-administration of the tachykinin-receptor antagonists with meclofenamic acid abolished the effects of bradykinin on trachea and on PIP while having no effect on responses to histamine. Each trace is representative of 5–6 experiments. See text for details of experimental design (see Tables 1 and 2; also see Fig. 7).

Fig. 7. Summary of the effects of histamine (A and B) and bradykinin (C and D) on TT (A and C) and PIP (B and D) in the absence (○) and presence of meclofenamic acid (3 mg/kg iv; ●) or meclofenamic acid and the tachykinin-receptor antagonists SR-140333, SR-48968, and SB-223412 (1 mg/kg iv each; ▲). Direct effects of bradykinin and histamine on trachea were prevented in all experiments by adding the histamine H1-receptor antagonist pyrilamine (1 µM) and the bradykinin B2-receptor antagonist FR-173657 (0.3 µM) to the tracheal perfusate. Each point is the mean ± SE of 5–10 experiments (see Tables 1 and 2). See text for details of experimental design.
airway reflexes (27, 27), the site of action and release of the tachykinins mediating bradykinin-induced reflexes is probably the brain stem. In further support of this hypothesis, ivc infusion of the tachykinin-receptor antagonist ZD-6021 in the present study completely abolished bradykinin-mediated, reflex tracheal contractions.

Histamine and bradykinin could evoke reflexes by altering pulmonary, bronchial, or systemic hemodynamics, or by inducing de novo synthesis of prostanoids or nitric oxide, which might then activate airway afferent nerves (10, 14, 19, 25, 39). We observed, however, that inhibiting the vasodilating effects of bradykinin and histamine with inhibitors of cyclooxygenase and nitric oxide synthase failed to prevent reflexes evoked by these autacoids. Furthermore, we could evoke tracheal reflexes similar to those induced by histamine using the bronchoconstrictor and vasoconstrictor LTD4. We assert, therefore, that the primary effects of histamine and bradykinin on airway autonomic tone proceed independent of their effects on the pulmonary or bronchial vasculature. Moreover, we conclude that, whereas prostanoids and NO may modulate responsiveness to autacoids, neither is necessary for activation of airway afferent nerves by histamine or bradykinin. Nevertheless, we did observe that a component of the response to bradykinin appeared to be dependent on prostaglandin synthesis. Because this prostaglandin-dependent reflex mimicked the effects of histamine on TT, we assert that the prostaglandins formed after bradykinin challenge activate airway afferent nerves indirectly, secondary to initiating airway obstruction. This observation along with the effects of propranolol reported here highlight the importance of experimental design when attempting to define specific mechanisms of autacoid-induced reflexes.

Reflex-mediated alterations in intrapulmonary airway TT and nonadrenergic noncholinergic parasympathetic nerve activity. There is little physiological or morphological evidence to suggest that parasympathetic nerve activity in the trachea differs substantially from that in the intrapulmonary airways (8, 16). It seems likely, therefore, that reflexes evoking alterations in TT reported in the present study are reflective of reflex-mediated alterations in parasympathetic tone throughout the airways. The effects of atropine and vagotomy on pulmonary responses evoked by histamine and bradykinin reported here and elsewhere (11, 13, 15, 44) are consistent with this hypothesis. The ease with which these reflexes are dissociated from the direct effects of these autacoids on airway mechanics with the use of the methods described here highlight the utility of our model system for studying reflex regulation of airway smooth muscle tone. By contrast, the inability to detect modulatory effects of atropine or vagotomy on many of the changes in PIP evoked in the present study highlight the insensitivity of this and any similar measurements for studying the role of the nervous system on airway mechanics.

Activation of either RARs or airway C fibers may initiate noncholinergic parasympathetic nerve-mediated bronchodilatation (17, 18, 46). In the present study, we observed that both bradykinin and histamine evoked reflex-mediated relaxations of the trachea. We also observed that intravenous administration of the nitric oxide synthase inhibitor L-NNA, which will inhibit parasympathetic nerve-mediated relaxations of airway smooth muscle, increased baseline TT. We speculate that noncholinergic parasympathetic nerves may tonically and reflexly modulate airway cholinergic nerve activity. Recent studies are consistent with this hypothesis (B. S. Kesler and B. J. Canning, unpublished observations).

Interactions between airway afferent nerves regulating airway TT. Our results do not rule out the possibility that, under certain experimental conditions, autacoids such as histamine and bradykinin and other stimuli that evoke airway reflexes do so through activation of multiple airway afferent subtypes. As mentioned above, histamine, when administered at high doses and thus producing profound bronchospasm, can activate airway C fibers, perhaps secondary to formation of bradykinin in challenged tissues (5, 24). Conversely, bradykinin, through its ability to induce peripheral tachykinin release, prostaglandin synthesis, and subtle contractions of airway smooth muscle, can alter dynamic lung compliance and thus activate airway RARs (14, 39). Serotonin has been reported to directly activate both airway C fibers and RARs (9, 30). Capsaicin might also activate multiple afferent subtypes innervating the airways (4, 19, 29, 35). Guinea pigs and rodents are, however, somewhat unique in their capacity for producing tachykinin-dependent, axon reflex-mediated airway effects (bronchospasm, plasma exudation, vasodilatation, mucus secretion; Ref. 25). We speculate, therefore, that many reflexes evoked by stimulation of airway C fibers are mediated indirectly, secondary to subsequent activation of RARs (4, 19, 40). Consistent with this hypothesis, peptide tachykinin-receptor antagonists, which are likely to have little, if any, CNS penetration, and bronchodilators, such as albuterol, counteract reflex effects (e.g., cough) evoked by putatively selective stimulants of airway C fibers (6, 32, 47). There are, however, several studies documenting central sites of action of the tachykinin-receptor antagonists in studies of airway reflexes (7, 26; present study).

SARs are relatively insensitive to chemical stimulation but are activated during inspiration and continuously activated during sustained lung inflation, elevated positive end-expiratory pressure or lung hyperinflation (41). Reflexes evoked by SAR activation are dependent on activation of GABAergic neurons in the brain stem (20). We have observed that central administration of the GABA_A-receptor antagonist bicuculline increases baseline cholinergic tone in the trachea (S. B. Mazzone, unpublished observations). Therefore, we speculate that SARs play an important modulatory role in regulating airway smooth muscle tone. This may be particularly relevant in studies using fixed-volume ventilation and aerosol challenges. Constriction and/or obstruction of the central airways
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