Multiple mechanisms of reflex bronchospasm in guinea pigs

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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 prostanoids 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.

Autacoids 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 stimuli 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 prostanoids 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 airwayafferent 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 gentle suction.

In all experiments described in this study, the Krebs carbonate 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 phen tolamine. 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 M) 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 abdominal 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 79E).

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 percent of the 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 μg/kg iv) or the bradykinin B2-receptor antagonist HOE-140 (1 mg/kg iv); 5) atropine (1 mg/ml) or histamine (1 μg/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 on Statview 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, phentolamine hydrochloride, and dl-propranolol hydrochloride were purchased from Sigma Chemical (St. Louis, MO). PGF$_{2\alpha}$ was purchased from Biomol (Plymouth, PA). Tri-methaphan camsylate 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). Bradykinin, ICI-198615, SR-48968 (saredutant), and SB-140 was provided by Aventis (Frankfurt, Germany). Stock solutions (10 mg/ml) of all drugs added to the tracheal perfusate were made in distilled water with the following exceptions: PGF$_{2\alpha}$ (10 mM) and indomethacin (30 mM) were dissolved in absolute ethanol, and ICI-198615 (10 mM), FR-173657 (1 mM) and bicuculline (10 nmol/µl) 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, SB-223412 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 µg/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 µg/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 µg·kg$^{-1}$·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, nebulated 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, nebulated histamine had no effect or evoked only decreases in TT. In the remaining three preparations, nebulated histamine induced modest increases in TT that were either followed or preceded by decreases in TT. Nebulated 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 µg/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, nebulated 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 µg/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 (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 H2-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 H2-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.

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, increasing 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 bronchoospasm. 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 bronechoconstrictor and pulmonary vasoconstrictor LTD4. In an animal pretreated intravenously with meclofenamic acid and L-NNA and intratracheally with the cysteinyl 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.
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. 6C, 7A, and 7B; 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. 6C, 7C, and 7D; 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). Subbrachial vagotomy, propranolol

![Graph](image_url)

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

<table>
<thead>
<tr>
<th></th>
<th>Pulmonary Insufflation Pressure</th>
<th>Tracheal Tone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum increasea</td>
<td>ED50, µg/kg</td>
</tr>
<tr>
<td>Histamine</td>
<td>10</td>
<td>195 ± 17e</td>
</tr>
<tr>
<td>+1 mg/kg atropine</td>
<td>6</td>
<td>155 ± 42</td>
</tr>
<tr>
<td>+1 mg/kg pyrilamine</td>
<td>3</td>
<td>0 ± 0d</td>
</tr>
<tr>
<td>+3 mg/kg meclofenamic acid</td>
<td>5</td>
<td>235 ± 28</td>
</tr>
<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>
</tr>
<tr>
<td>+3 mg/kg meclofenamic acid (iv) and 3.3 nmol/min (icv) ZD-6021</td>
<td>5</td>
<td>185 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE. ED50, dose of histamine required to produce 50% of the maximum response; icv, intracerebroventricular.

a,b Maxima represent percent increase in pulmonary insufflation pressure and percent maximum contraction, respectively. c Significantly different from ED50 of histamine for increasing tracheal tone, P < 0.02. d Significantly different from histamine control, P < 0.02.

*Significantly different from corresponding bradykinin value (Table 2), P < 0.001.

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(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 (22061%), whereas 5 mg/kg L-NNA combined with meclofenamic acid increased baseline TT (80650%; 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

**Table 2. Pharmacology of intravenous bradykinin-mediated airway effects in guinea pigs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Maximum increasea</th>
<th>ED₅₀, nmol/kg</th>
<th>Maximum increaseb</th>
<th>ED₅₀, nmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>10</td>
<td>95 ± 19e</td>
<td>0.3 ± 0.1</td>
<td>41.4 ± 5.8e</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>+1 mg/kg atropine</td>
<td>5</td>
<td>74 ± 25</td>
<td>0.7 ± 0.03e</td>
<td>3.1 ± 1.2d</td>
<td>&gt;2.0d</td>
</tr>
<tr>
<td>+1 mg/kg HOE-140</td>
<td>3</td>
<td>0 ± 0d</td>
<td>&gt;2.0d</td>
<td>0 ± 0d</td>
<td>&gt;2.0d</td>
</tr>
<tr>
<td>+1 mg/kg SR-140333, SR-48968, SB-223412</td>
<td>10</td>
<td>77 ± 11</td>
<td>0.5 ± 0.2</td>
<td>41.4 ± 5.6</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>+3 mg/kg meclofenamic acid</td>
<td>6</td>
<td>8 ± 4d</td>
<td>&gt;2.0d</td>
<td>25.5 ± 3.5c</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>+3 mg/kg meclofenamic acid and 1 mg/kg each SR-140333, SR-48968, SB-223412</td>
<td>5</td>
<td>0 ± 0d</td>
<td>&gt;2.0d</td>
<td>3.3 ± 1.1d</td>
<td>&gt;2.0d</td>
</tr>
<tr>
<td>+3 mg/kg meclofenamic acid (iv) and 3.3 nmol/min (icv) ZD-6021</td>
<td>5</td>
<td>0 ± 0d</td>
<td>&gt;2.0d</td>
<td>3.0 ± 2.2d</td>
<td>&gt;2.0d</td>
</tr>
</tbody>
</table>

Values are means ± SE. ED₅₀, dose of bradykinin required to produce 50% of the maximum response. a,b Maximum percent increase in pulmonary insufflation pressure and percent maximum contraction, respectively. c Significantly different from bradykinin control, P = 0.05. d Significantly different from bradykinin control, P < 0.01. e Significantly different from corresponding histamine value, P < 0.001 (Table 1).

Fig. 4. Representative traces showing the effects of propranolol (B; 1 mg/kg iv) on responses evoked by intravenously administered histamine. A: control conditions. See text for details of experimental design (propranolol (2 μM), pyrilamine (1 μM), and FR-173657 (0.3 μM) were always present in the tracheal perfusate). Propranolol induced a leftward shift in the concentration-response curve for histamine-induced increases in PIP and TT. Thus 2 μg/kg histamine failed to influence either TT or PIP under control conditions, whereas, after intravenous administration of propranolol, 2 μg/kg histamine increased TT and PIP by 50 ± 26 and 31 ± 9% over baseline, respectively (n = 5; P < 0.05). By contrast, propranolol was without effect on tracheal reflexes evoked by intravenously administered 0.1 nmol/kg bradykinin, which increased TT by 34 ± 17% (n = 5) in the absence of propranolol and 45 ± 19% (n = 6) in the presence of propranolol, respectively (P > 0.1).
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...
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 prostanoid synthesis. Because this prostanoid-dependent reflex mimicked the effects of histamine on TT, we assert that the prostanoid released was a consequence of histamine challenge. Therefore, to initiate airway obstruction, we speculate that noncholinergic parasympathetic reflex-mediated alteration 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. Cannon, 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, prostanoid 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 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...
during fixed volume ventilation will increase distension of the peripheral airways, thereby increasing discharge of stretch receptors innervating the intrapulmonary airways. The effects of increased positive expiratory pressure on baseline TT reported here and elsewhere (22, 28, 37) and the effects of positive end-expiratory pressure on reflexes evoked by histamine reported in the present study are consistent with this hypothesis. The unreliable effects of nebulized histamine on TT (relative to its effects when administered intravenously) further suggest the presence of an inhibitory reflex regulating TT. Finally, the data indicate that reflexes may require synergistic interactions between airwayafferent nerves. Unlike airways C fibers, which are generally quiescent during tidal breathing, RARs and SARs are sporadically active during the respiratory cycle (25, 40, 41). Ongoing RAR activity during eupneic breathing controls baseline cholinergic tone in the airways (22). Consequently, RAR-dependent reflexes may be produced by increasing their baseline activity or by increasing their sensitivity to the mechanical forces of respiration. Conversely, reflexes evoked by airway C fibers will be overlaid with the ongoing afferent activity of mechanically sensitive afferents innervating the airways. Modulation of SAR activity or alterations of synaptic transmission in the CNS could also produce profound alterations parasympathetic tone.

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