Effects of human eosinophil granule-derived cationic proteins on C-fiber afferents in the rat lung

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Effects of human eosinophil granule-derived cationic proteins on C-fiber afferents in the rat lung. J Appl Physiol 91: 1318–1326, 2001.—Experiments were performed to test the hypothesis that human eosinophil granule-derived cationic proteins stimulate vagal C-fiber afferents in the lungs and elicit pulmonary chemoreflex responses in anesthetized Sprague-Dawley rats. Intratracheal instillation of eosinophil cationic protein (ECP; 1–2 mg/ml, 0.1 ml) consistently induced an irregular breathing pattern, characterized by tachypnea (change in breathing frequency of 44.7%) and small unstable tidal volume (VT). The tachypnea, accompanied by decreased heart rate and arterial blood pressure, started within 30 s after the delivery of ECP and lasted for >30 min. These ECP-induced cardiorespiratory responses were completely prevented by perineural capsaicin treatment of both cervical vagi, which selectively blocked C-fiber conduction, suggesting the involvement of these afferents. Indeed, direct recording of single-unit activities of pulmonary C-fibers further demonstrated that the same dose of ECP evoked a pronounced and sustained (>30-min) stimulatory effect on pulmonary C-fibers. Furthermore, the sensitivity of these afferents to lung inflation was also markedly elevated after the ECP instillation, whereas the vehicle of ECP administered in the same manner had no effect. Other types of eosinophil granule cationic proteins, such as major basic protein and eosinophil peroxidase, induced very similar respiratory and cardiovascular reflex responses. In conclusion, these results show that eosinophil granule-derived cationic proteins induce a distinct stimulatory effect on vagal pulmonary C-fiber endings, which may play an important role in the airway hyperresponsiveness associated with eosinophil infiltration in the airways.

asthma; airway hyperresponsiveness; airway inflammation; pulmonary C-fibers; tachypnea

IT HAS BEEN WELL DOCUMENTED that a distinct association between the increase in eosinophils in the bronchoalveolar lavage fluid and the late-phase asthmatic reactions exists in humans (12, 26, 30). Degranulation of eosinophils leads to the release of four major types of low-molecular-weight, highly cationic and basic proteins: major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN) (1, 14). These cationic proteins are believed to play a key role in the eosinophil infiltration-induced airway mucosal injury and pathogenesis of asthma (13, 18, 30). Previous studies have demonstrated that intratracheal instillation of these eosinophil granule-derived cationic proteins, such as MBP and ECP, induces bronchial hyperresponsiveness in a number of animal species (11, 17, 18). Similar effects can also be induced by the intratracheal administration of synthetic cationic proteins such as poly-L-lysine (11). Furthermore, the airway hyperresponsiveness and plasma protein extravasation resulting from the intratracheal challenge with poly-L-lysine in the rat are mediated through the release of tachykinins, presumably from C-fiber sensory terminals in the airways and lungs (10). Thus previous studies suggest a possible involvement of these sensory nerves in the bronchial hyperresponsiveness induced by the cationic proteins. Indeed, stimulation of bronchopulmonary C-fibers is known to cause bronchoconstriction, hypersecretion of mucus, and other pronounced effects on airway functions, which are mediated through cholinergic reflexes and tachykinergic mechanisms (7, 24, 25, 28). Furthermore, increasing evidence indicates that pulmonary C-fiber afferents play an important role in the manifestation of airway hyperresponsiveness associated with airway inflammation (24, 29). However, whether these cationic proteins exert a stimulatory and/or sensitizing effect on the C-fiber endings in the lungs is unknown, and the direct electrophysiological evidence in support of such effects has not been established. Moreover, we reason that if stimulation of pulmonary C-fiber endings is involved, administration of these cationic proteins should elicit the respiratory and cardiovascular responses exhibiting the characteristics of pulmonary chemoreflexes (7, 24) in spontaneously breathing animals. Hence, the purpose of this study was fourfold: 1) to determine the cardiorespiratory changes induced by intratracheal instillation of all four types of eosinophil granule-derived cationic proteins, 2) to investigate the role of vagal bronchopulmonary C-fiber afferents in eliciting these responses, 3) to determine the effect of these cationic proteins on the electrophysiological activities of vagal pulmonary C-fibers further demonstrated that the same dose of ECP evoked a pronounced and sustained (>30-min) stimulatory effect on pulmonary C-fibers. Furthermore, the sensitivity of these afferents to lung inflation was also markedly elevated after the ECP instillation, whereas the vehicle of ECP administered in the same manner had no effect. Other types of eosinophil granule cationic proteins, such as major basic protein and eosinophil peroxidase, induced very similar respiratory and cardiovascular reflex responses. In conclusion, these results show that eosinophil granule-derived cationic proteins induce a distinct stimulatory effect on vagal pulmonary C-fiber endings, which may play an important role in the airway hyperresponsiveness associated with eosinophil infiltration in the airways.

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C-fibers, and 4) to investigate whether the sensitivities of these afferents to mechanical and chemical stimuli are enhanced by the administration of these eosinophil proteins. The last two aims focused on the effects of ECP and MBP, because these cationic proteins evoked more consistent reflex responses in our preliminary experiments.

METHODS

Animal Preparation

The procedures described below were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and also were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Sprague-Dawley rats were anesthetized with an intraperitoneal injection of α-chloralose (100 mg/kg; Sigma Chemical) and urethane (500 mg/kg; Sigma Chemical) dissolved in a 2% borax solution; supplemental doses of the same anesthetics were injected intravenously whenever necessary to maintain abolition of pain reflex elicited by paw pinch. The left jugular vein was cannulated with the tip of the catheter positioned slightly above the right atrium for injections. Body temperature was maintained at ~36°C throughout the experiment with a servo-temperature controller and a heating pad placed under the animal. A short tracheal cannula was inserted just below the larynx via a tracheotomy, and tracheal pressure (Ptr) was measured (model MP 45-28, Validyne) via a side port of the tracheal cannula.

Measurements of Respiratory and Cardiovascular Responses

The effects of intratracheal instillations of ECP, MBP, EPO, and EDN on respiration, arterial blood pressure (ABP), and heart rate (HR) were investigated in the rats breathing spontaneously in a supine position. Respiratory flow was measured with a heated pneumotachograph and a differential pressure transducer (model MP45-14, Validyne) and integrated to give tidal volume (Vt). The femoral artery was cannulated for recording ABP and HR and for collecting blood samples for blood gas measurements (model 1306, Instrumentation Laboratory). Respiratory frequency, Vt, minute ventilation, ABP, and HR were analyzed (model TS-100, Biocybernetics) on a breath-by-breath basis by an online computer.

Recording of Pulmonary C-Fiber Activity

Pulmonary C-fiber activities were recorded in anesthetized, artificially ventilated rats; stroke volume and frequency of the respirator were set at 8–10 ml/kg and 45 breaths/min, respectively. After a midline thoracotomy, both vagus nerves were ligated just above the diaphragm to eliminate afferent signals arising from lower visceral organs. The opening of the thorax was covered by a sheet of polyethylene film to keep the lung moist, and the expiratory outlet of the respirator was placed under 3 cmH2O pressure to maintain a near-normal functional residual capacity. The right cervical vagus nerve was sectioned as rostrally as possible, and the caudal end of the cut vagus was placed on a small dissection platform and immersed in a trough of mineral oil held by tethered cervical skin. A thin filament was teased away from the desheathed nerve trunk and placed on a platinum-iridium miniature electrode; action potentials were monitored by an audio monitor and displayed on an oscilloscope. The thin filament was further split until a single unit was electrically isolated judging from the pattern of its discharge. Pulmonary C-fibers usually have a sparse or no baseline activity but can be mildly activated by hyperinflation of the lungs (3–4 Vt, peak Ptr >35 cmH2O (20). After the activity of a suspected C-fiber was identified by lung inflation, capsaicin (0.5–1.0 μg/kg) was injected via jugular venous catheter into the right atrium; only the fibers that responded to capsaicin within 1 s after the injection were studied. In addition, the receptor field of each fiber was identified by gentle palpation or probing with a blunt-ended glass rod; fibers that could not be localized in the lungs or airways were excluded from the study. Conduction velocities of ~31% of the fibers were measured as described previously (19). Action potentials, Ptr, and ABP were recorded on a videocassette recorder (model 500H, Pacer/Vetter, Los Angeles, CA), and fiber activity was analyzed by the computer (model TS-100, Biocybernetics) for each 0.5-s interval.

Experimental Protocols

Three series of experiments were carried out. Respiratory and cardiovascular responses to intratracheal instillations of ECP, MBP, EPO, and EDN. A small volume (0.1 ml) of ECP (1–2 mg/ml) was instilled into the lung via the tracheal cannula, then the lungs were hyperinflated (5 ml) twice to facilitate the dispersion of ECP to the lung periphery; the instillation procedure required the rat to be disconnected from the recording apparatus for a brief duration (~10 s). Baseline ventilatory and cardiovascular parameters were measured before and continuously for >1 h after the ECP instillation in seven rats. Control experiments were carried out in a separate group of rats (n = 6) following the same protocol, except ECP was replaced by its vehicle [0.1 ml intratracheally (it)]. In another group of rats (n = 6), the same dose of ECP was injected intravenously to investigate whether the route of delivery is critical to the observed responses. Likewise, the effects of MBP (1–2 mg/ml, 0.1 ml it, n = 6) and its vehicle (n = 6), EPO (4–5 mg/ml, 0.1 ml it, n = 6), and EDN (1–2 mg/ml, 0.1 ml it, n = 4) were studied in four more groups of rats following the protocols described above.

Role of vagal bronchopulmonary C-fibers. To assess the role of C-fiber afferents, we used the method of perineural capsaicin treatment (PNCT) of both cervical vagus nerves to selectively block the neural conduction in these fibers as described in our previous study (22). Briefly, cotton strips soaked in capsaicin solution (250 μg/ml) were wrapped around a 2- to 3-mm segment of the isolated cervical vagus nerves for 15–20 min and then removed. Our criterion for a successful PNCT was a complete abolition of the reflex responses to capsaicin injection (0.5–1.0 μg/kg iv). The responses to intratracheal instillations of ECP were studied within 15 min after completion of the PNCT and then again 20 min later (n = 6); in the previous study, we showed that the blocking effect of this treatment lasted for >60 min (22).

Effects of ECP and MBP on vagal pulmonary C-fibers. Afferent activities of single-unit pulmonary C fibers were measured before and 2, 10, 20, 30, and 60 min after the intratracheal instillation of ECP (1.0–2.0 mg/ml, 0.1 ml) in open-chest, artificially ventilated rats (n = 12). We also investigated the effects of ECP on pulmonary C-fiber sensitivities to mechanical and chemical stimuli in the same fibers. Mechanical stimulation was applied by hyperinflation of the lungs separately with two different pressures (15 and 30 cmH2O Ptr) that was maintained constant for 10 s. Capsaicin (0.5–1.0 μg/kg iv) was chosen as the chemical stimulus.
of pulmonary C-fibers because of its potent and selective stimulatory effect on pulmonary C-fibers. Pulmonary C-fiber responses to lung inflations and capsaicin challenge were determined before and 10, 30, and 60 min after the intratracheal instillation of ECP in 12 rats; this protocol was designed to avoid tachyphylaxis due to repeated challenges of capsaicin. For comparison, control experiments in which the ECP vehicle was administered in the same manner were carried out in a separate group of rats (n = 7). Protocols identical to those described above for ECP were followed to study the effects of MBP (n = 11) and its vehicle (n = 6) on pulmonary C-fibers and their sensitivities to lung inflation and capsaicin in two other groups of rats.

Materials

Human eosinophils were obtained by cytapheresis of patients with marked eosinophilia. Eosinophil granules were prepared from eosinophils as described previously (1) and were solubilized in 0.01 M HCl before fractionation on Sephadex G-50. Fractions rich in EDN and ECP were subsequently purified on heparin Sepharose (15), and EPO was purified on carboxymethyl Sepharose (6). MBP was contained in the third peak from the Sephadex G-50 column fractionation and was likely contaminated with a small quantity of the MBP homolog (27). A pool of prevoid volume fractions from the Sephadex G-50 column was used as the buffer control for MBP. ECP, EPO, and EDN were stored at 280°C and diluted in vehicles of isotonic saline or 0.53 PBS 10.1 M NaCl before use. MBP was stored (−80°C) in 0.025 M acetate buffer and diluted with 0.15 M NaCl or PBS before use. Stock solution of capsaicin (Sigma Chemical; 500 μg/ml) was prepared in a vehicle of 10% Tween 80, 10% ethanol, and 80% saline; solution of the desired concentration for injection or perineural treatment was prepared daily with isotonic saline dilution.

Statistical Analysis

A one- or two-way repeated-measures analysis of variance was used for the statistical analysis. For the latter, one factor was the treatment effect (e.g., ECP vs. vehicle) and the other factor was the time effect (e.g., responses at different time points after ECP). When the two-way analysis of variance showed a significant interaction, pairwise comparisons were made with a post hoc analysis (Newman-Keuls test). Values are means ± SE. P < 0.05 was considered significant.

RESULTS

Three study series were carried out in a total of 83 rats (310–455 g).

Study 1

In anesthetized, spontaneously breathing rats, intratracheal instillation of a bolus of ECP (1.0–2.0 mg/ml, 0.1 ml) induced a distinctly irregular breathing pattern characterized by a high respiratory rate and a small, unstable VT (Fig. 1). The tachypnea, accompanied by decreased HR and ABP (Figs. 1 and 2), started within 30 s after the instillation and was sustained for >30 min; respiratory frequency increased from 68.0 ± 5.5 breaths/min at control to 98.3 ± 14.1 breaths/min (P < 0.05, n = 7) at 10 min after the instillation of ECP, and VT decreased from 2.08 ± 0.11 to 1.68 ± 0.2 ml (P < 0.05; Fig. 2). Concomitantly, HR decreased from 344.3 ± 15.7 to 288.6 ± 19.0 beats/min (P < 0.05), and ABP decreased from 114.7 ± 4.6 to 104.7 ± 6.2 mmHg (P < 0.05) after the ECP challenge (Figs. 2 and 3). There was no significant change in minute ventilation or arterial blood gases at the same time points after the ECP challenge. The irregular breathing pattern and reduced HR and blood pressure gradually subsided after >90 min in the three rats whose recovery time was measured (Fig. 1).

These responses were not caused by the procedure of intratracheal instillation, because instillation with the vehicle of ECP had no effect or evoked only a very mild irregular breathing pattern that returned to control within 30–45 s after the administration. There were no differences in respiratory frequency, HR, and ABP be-

Fig. 1. Effect of intratracheal instillation of eosinophil cationic protein (ECP, 200 μg in 0.1 ml) on baseline breathing pattern and arterial blood pressure (ABP) in an anesthetized rat (395 g). A: before ECP. B, C, and D: 10, 30, and 120 min, respectively, after ECP. VT, tidal volume.
between before and 10 min after the administration of vehicle (Fig. 3). Furthermore, intravenous injection of a higher \((2\times)\) dose of ECP failed to cause any detectable change in breathing pattern, HR, or ABP (Fig. 3).

In a separate group of rats, administration of MBP \((1–2\ \text{mg/ml}, 0.1\ \text{ml it})\) induced respiratory and cardiovascular responses very similar to those evoked by ECP; distinct increase in respiratory frequency and decrease in HR started within 30 s after the instillation and lasted for \(>30\ \text{min}\) \((n = 6;\ \text{Fig. 3})\), whereas neither the vehicle of MBP (acetate buffer, 0.1 ml it) nor intravenous injection of a double dose of MBP caused any significant changes at the same time points.

Intratracheal instillation of EPO \((4–5\ \text{mg/ml}, 0.1\ \text{ml})\) elicited respiratory and cardiovascular responses that were similar to but weaker than those evoked by ECP or MBP (Fig. 3); a higher dose of EPO was used because of its larger molecular weight \((\sim 70,000\ \text{for EPO and} < 21,000\ \text{for the other 3 cationic proteins})\). Administration of EDN \((1–2\ \text{mg/ml}, 0.1\ \text{ml it})\) failed to evoke any significant changes (Fig. 3).

**Study 2**

There was no detectable change in the baseline \(V_T\), respiratory frequency, ABP, or HR after PNCT of both cervical vagi (Fig. 2). PNCT of both vagi did not significantly alter the reflex apneic responses to lung inflation \((6\ \text{cmH}_2\text{O} \text{Ptr})\) but completely abolished the reflex responses to capsaicin injection \((0.5–1.0\ \mu\text{g/kg iv})\), indicating a selective blockade of the C-fiber afferents. In sharp contrast to that displayed in the control animals (study 1), the tachypnea and decreased HR and blood pressure caused by the intratracheal administration of ECP were completely prevented by PNCT of both vagi (Figs. 2 and 3), suggesting an important role of vagal C-fibers in eliciting these reflex responses.

**Study 3**

A total of 36 pulmonary C-fibers were studied in 30 open-chest, artificially ventilated rats. The location of each receptor was identified: 11 were found in the upper lobe, 10 in the middle lobe, 14 in the lower lobe, and 1 in the accessory lobe of the right lungs. The conduction velocity was measured in 11 fibers and ranged from 0.93 to 1.75 m/s \((1.18 \pm 0.07\ \text{m/s})\).

Intratracheal instillation of ECP \((1.0–2.0\ \text{mg/ml}, 0.1\ \text{ml})\) evoked a distinct and long-lasting stimulatory effect on pulmonary C-fibers \((n = 12);\) fiber activity increased markedly from a baseline of \(0.01 \pm 0.01\ \text{impulses/s (imp/s)}\) to a peak of \(0.24 \pm 0.05\ \text{imp/s (20-s average)}\) at \(\sim 2\ \text{min after the challenge (Figs. 4 and 5A).}\) In general, pulmonary C-fibers did not respond immediately to the instillation of ECP, but their activities began to increase after a delay of \(10–30\ \text{s}\) and remained significantly higher than control \((P < 0.05)\) even after 30 min. In contrast, intratracheal instilla-

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**Fig. 2. Role of vagal C-fibers in the respiratory and cardiovascular responses to ECP. A: effect of intratracheal instillation of ECP \((100–200\ \mu\text{g})\) on baseline respiratory frequency \((f), V_T, \text{ABP, and heart rate (HR)}\) in control rats \((n = 7).\) B: effect of the same dose of ECP in another group of rats \((n = 6)\) that received prior perineural capsaicin treatment of both vagi.**
tion of the vehicle of ECP in the same manner did not cause any stimulatory effect on seven other pulmonary C-fibers at any of the same time points (Fig. 5A). In 3 of the 12 fibers that responded to ECP, the receptor discharge after the ECP instillation was synchronous with the inspiratory cycles of the respirator (Fig. 4). Instillation of ECP did not elicit significant changes in HR and ABP in some of the animals (Fig. 4), probably because the right vagus was sectioned for recording in this study. Intravenous injection of a higher dose (2 ×) of ECP failed to cause any stimulatory effect on three of the pulmonary C-fibers studied.

ECP instillation also markedly enhanced the sensitivity of pulmonary C-fiber afferents to lung inflation (Fig. 6); receptor responses to low- (15 cmH₂O) and high-pressure (30 cmH₂O) inflations were significantly elevated at 10 min after the ECP challenge (P < 0.05, n = 12; Fig. 7), and this sensitizing effect of ECP lasted for >30 min. In contrast, administration of the ECP vehicle did not alter the responses to low- or high-pressure inflation (n = 7; Fig. 7). Responses to right atrial injection of capsaicin (0.5–1.0 μg/kg) were also clearly elevated in some (5 of 12) of these fibers, but the group data as a whole failed to reach a statisti-
cally significant difference (P > 0.05) because of a large variability of the responses between individual fibers.

Similar but less sustaining stimulatory effects on pulmonary C-fibers were also induced by intratracheal instillation of MBP (1.0–2.0 mg/ml, 0.1 ml) in a separate group of rats (Fig. 5B). MBP evoked a clear increase in the baseline fiber activity, which increased from a control of 0.01 ± 0.01 imp/s to a peak of 0.41 ± 0.05 imp/s (20-s average; P < 0.05, n = 11) in ~2 min after the instillation and gradually declined but remained significantly higher than control at ~10 min (Fig. 5B). In contrast, instillation of the vehicle of MBP in the same manner did not cause any stimulatory effect on six other pulmonary C-fibers (Fig. 5B). The same dose of MBP also induced a mild increase (P < 0.05, n = 11) of the sensitivity to low- and high-pressure lung inflations at 10 min after the instillation, but the enhanced responses were relatively short lasting and returned to control within 30 min.

DISCUSSION

Our results clearly showed that intratracheal instillation of eosinophil granule-derived cationic proteins induced irregular breathing pattern and lower HR and ABP in anesthetized rats; these effects started within 30 s and lasted for >30 min. These effects could be prevented by PNCT of both vagi, which selectively blocks the conduction of C-fibers, suggesting an involvement of vagal C-fiber afferents. Indeed, it is well documented that pulmonary chemoreflexes, characterized by a triad of tachyphnea (often preceded by an apnea), bradycardia, and hypotension, are elicited by the stimulation of pulmonary C-fibers with right atrial or intravenous bolus injection of chemical irritants (7, 24). Results obtained from our electrophysiological recording experiment further demonstrated that instillation of ECP or MBP evoked a distinct and long-lasting stimulatory effect on vagal bronchopulmonary C-fibers. Furthermore, these cationic proteins also markedly augmented the sensitivities of these afferents to lung inflation. These enhanced responses appear to result specifically from the effects generated by these cationic proteins and not from the procedures of intratracheal instillation, because the instillation of the vehicles of these proteins, except during the brief (<45-s) initial period, did not evoke any detectable effects on the cardiorespiratory reflex responses or on the pulmonary C-fiber afferents (Figs. 3 and 5). Although pulmonary C-fibers are known to have polymodal sensitivities, they are relatively insensitive to lung inflation in control animals (19). Hence, the enhanced sensitivity of these afferents to lung inflation observed in this study may have significant implications, because an increase in VT occurs commonly under normal physiological conditions. For example, VT increases as ventilation increases during exercise or in response to hypoxia; in patients with eosinophilic airway inflammation, the augmented discharge of pulmonary C-fibers induced by ECP and MBP may therefore contribute, in part, to the dyspneic sensation and bronchoconstriction under those conditions.

Morphological evidence shows that ~75% of theafferent fibers in the vagal branches arising from the respiratory tract are C-fibers (3), and these vagal C-fiber afferents innervate the entire respiratory tract, ranging from large conducting airways to the lung parenchyma (7, 24). Stimulation of pulmonary C-fiber afferents is known to elicit a number of reflex responses mediated through the central nervous system and/or autonomic nervous system, including bronchoconstriction, mucus hypersecretion, coughing, dyspneic sensation, and bronchial vasodilation (7, 24). In addition, several sensory neuropeptides such as tachykinins (e.g., substance P, neurokinin A) and calcitonin gene-related peptide are synthesized in the cell bodies of pulmonary C-fibers and released locally from the sensory terminals on stimulation. These peptides are known to act on a number of effector cells (e.g., airway smooth muscles, cholinergic ganglia, inflammatory cells, mucous glands) and produce potent local effects.
such as bronchoconstriction, extravasation of macromolecules, and edema of airway mucosa in various species including humans (25, 28). Taken together, it seems reasonable to hypothesize that when eosinophils infiltrate into the airways (e.g., during airway inflammation and anaphylaxis), the secretion of these cationic proteins may activate and/or sensitize the pulmonary C-fiber endings and play a part in the manifestation and development of the bronchial hyperreactivity, dyspneic sensation, and cough (7, 24).

It is well documented that instillation of purified MBP or synthetic cationic proteins into the trachea can induce airway hyperresponsiveness in a number of animal species (11, 17, 18). Furthermore, the effect of these proteins can be prevented by pretreatment with the selective antagonist of neurokinin-1 receptor, suggesting a critical role of endogenously released substance P (10). Other studies showed that these cationic proteins may act directly or indirectly via the endogenous tachykinins on the muscarinic M2 receptors on the cholinergic postganglionic nerves and cause dysfunction of these receptors, which in turn leads to airway hyperresponsiveness (8, 21). Taken together, these previous studies suggest that the cationic protein-induced airway hyperresponsiveness involves the tachykinins (8, 10). The present study has provided the direct evidence of stimulatory effects on respiratory reflexes and on pulmonary C-fiber afferent activities. In comparison, the cardiorespiratory responses to EPO administered at approximately the same molar doses were relatively less pronounced, whereas EDN failed to induce significant changes. Whether the effects of ECP and MBP observed in this study are related to certain properties of the individual proteins, in addition to their charge-dependent action, remains unclear.

The delivery of these cationic proteins via the airways appears to be critical in evoking the resulting

![Fig. 6. Effect of intratracheal instillation of ECP (100 μg in 0.1 ml) on responses to lung inflations of a C-fiber arising from ending located in the lower lobe of right lung in an anesthetized, open-chest, artificially ventilated rat (370 g). Conduction velocity of this fiber was 0.93 m/s. A and B: before ECP. C and D: ~10 min after ECP. E and F: ~60 min after ECP. A, C, and E: low-pressure (15 cmH₂O Pt) inflation. B, D, and F: high-pressure (30 cmH₂O Pt) inflation. See Fig. 4 legend for further explanation.](http://jap.physiology.org/)
effects, because intravenous injection of a much higher dose of ECP or MBP failed to elicit any detectable effect on cardiorespiratory parameters or pulmonary C-fiber activity. Previous investigators have suggested that an interaction between the cationic proteins and the epithelial cells plays an integral role in the airway hyperresponsiveness induced by these proteins (5, 13, 18). Their conclusion is supported by the fact that these cationic proteins are known to have profound effects on the airway epithelium; for example, MBP and ECP can cause epithelial damage in the airways (13, 18). However, it is unlikely that mucosa injury is primarily responsible for the effects of these cationic proteins on pulmonary C-fibers, because the responses observed in this study were transient and reversible within 60 min (Figs. 4 and 5). On the other hand, the cationic charges carried by these proteins are expected to facilitate their binding to the anionic surfaces of epithelial cells and other potential target cells in the airway mucosa, which may then evoke a subsequent release of various mediators. The response of pulmonary C-fiber afferents to the bolus administration of ECP or MBP exhibited a latency longer than that expected from a direct action on the sensory terminals. In fact, the relatively long latency of the response seems to correspond to the general pattern of slow release of endogenous mediators. Moreover, ECP, MBP, and other eosinophil granule-derived cationic proteins have been shown to cause release of prostaglandins from epithelial cells (31) and histamine from mast cells (32). These cationic proteins are also known to activate kallikrein and generate bradykinin (9). Some of these autacoids (e.g., histamine, prostaglandin E2) can exert a profound sensitizing and stimulatory effect on the C-fiber sensory terminals (19, 23, 24), which are located immediately below or between the airway epithelial cells (2, 4). Hence, it seems reasonable to assume that these and other endogenous mediators may collectively contribute to the observed effect of these cationic proteins on C-fiber terminals.

Our results show that the stimulatory effects of ECP on pulmonary C-fiber afferents gradually decreased; the peak response declined to ~30% at 30 min after the challenges. The cardiorespiratory reflex responses to ECP (tachypnea and bradycardia) also showed progressive recovery, but the responses seemed to be sustained longer than that of the C-fiber discharge. The reason for this discrepancy in recovery time is not known. One possibility is that the initial stimulation of the C-fiber endings by these proteins and the release of tachykinins may have triggered a cascade of neurogenic inflammatory reactions (25, 28), which contribute to the subsequent lingering systemic effects. Furthermore, to avoid any experimental artifacts generated by respiratory movements, the pulmonary C-fiber activity was recorded in animals artificially ventilated, whereas the cardiorespiratory reflex responses were studied in spontaneously breathing animals. PTR, respiratory flow, and volume are very different between these two experimental preparations. Whether these differences affected the dispersion of cationic proteins in the tracheobronchial tree and influenced the severity of their effects and, consequently, the recovery time remains to be determined.

In conclusion, these results show that intratracheal instillation of human eosinophil granule cationic proteins induces a pronounced and sustaining stimulatory effect on vagal pulmonary C-fiber endings and enhances their sensitivity to lung inflation in anesthetized rats. Although the mechanism underlying the C-fiber activation is not known, it is probably related to an interaction between the cationic charge carried by these proteins and the airway mucosa, leading to a subsequent release of inflammatory mediators. In view of the increasing evidence of the profound influence of the pulmonary C-fiber activation on various airway functions, we suggest that the effects of the cationic proteins on these sensory terminals play an important role in the manifestation of airway hyperresponsiveness associated with eosinophil infiltration in the airways.
REFERENCES


