Effect of synthetic cationic protein on mechanoexcitability of vagal afferent nerve subtypes in guinea pig esophagus

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Yu S, Ouyang A. Effect of synthetic cationic protein on mechanoexcitability of vagal afferent nerve subtypes in guinea pig esophagus. Am J Physiol Gastrointest Liver Physiol 301: G1052–G1058, 2011. First published September 29, 2011; doi:10.1152/ajpgi.00015.2011.—Eosinophilic esophagitis is characterized by increased infiltration and granulomatous changes of eosinophils in the esophagus. Whether eosinophil-derived cationic proteins regulate esophageal sensory nerve function is still unknown. Using synthetic cationic protein to investigate such effect, we performed extracellular recordings from vagal nodose or jugular neurons in ex vivo esophageal-vagal preparations with intact nerve endings in the esophagus. Nerve excitabilities were determined by comparing action potentials evoked by esophageal distensions before and after perfusion of synthetic cationic protein poly-L-lysine (PLL) with or without pretreatment with poly-L-glutamic acid (PLGA). We neutralized cationic charges of PLL. Perfusion with PLL did not evoke action potentials in esophageal nodose C fibers but increased their responses to esophageal distension. This potentiation effect lasted for 30 min after washing out of PLL. Pretreatment with PLGA significantly inhibited PLL-induced mechanohyperexcitability of esophageal nodose C fibers. In esophageal nodose Aβ fibers, perfusion with PLL did not evoke action potentials. In contrast to nodose C fibers, both the spontaneous discharges and the responses to esophageal distension in nodose Aβ fibers were decreased by perfusion with PLL, which can be restored after washing out PLL for 30–60 min. Pretreatment with PLGA attenuated PLL-induced decrease in spontaneous discharge and mechanoeccitability of esophageal nodose Aβ fibers. In esophageal jugular C fibers, PLL neither evoked action potentials nor changed their responses to esophageal distension. Collectively, these data demonstrated that synthetic cationic protein did not evoke action potential discharges of esophageal vagal afferents but had distinctive sensitization effects on their responses to esophageal distension.

sensory afferent; cationic protein; peripheral sensitization

Eosinophilic esophagitis (EoE) has recently emerged as a significant esophageal disorder affecting all age groups. Patients usually present with dysphagia and heartburn, with pathological finding of increased numbers of eosinophils in esophageal mucosal biopsies (19). Whether and how such eosinophilic infiltration causes these symptoms is still unknown. In allergic or inflammation conditions, eosinophils may migrate from circulation and infiltrate peripheral tissues and release cationic granule proteins, lipid mediators, and cytokines. Eosinophil-derived cationic granule proteins are toxic to parasitic organisms and may also damage normal tissues. Sensory afferent nerves sense changes in intercellular microenvironment in innervated peripheral tissue. A variety of inflammatory mediators released in their proximity regulate their excitabilities (18). Thus eosinophil-released cationic proteins may play a crucial role in esophageal sensory dysfunction in EoE. Currently, little is known about the effect of eosinophil-derived granule proteins on esophageal sensory afferent functions.

Eosinophil-specific proteins mainly include major basic protein and eosinophil cationic protein. These two cationic polypeptides have no known enzymatic activities, but both are capable of damaging the surrounding tissues. Previous studies demonstrated that both eosinophil cationic proteins (13) and major basic protein (7, 13) sensitize airway vagal C fibers to induce airway hyperresponsiveness. This sensitization effect is long lasting and can be prevented by pretreatment with a polyanion such as poly-L-glutamic acid (PLGA) or poly-L-aspartic acid to neutralize cationic charges. Once sensitization occurs, applying PLGA or poly-L-aspartic acid cannot reverse this effect. Whether these cationic proteins activate and/or sensitize esophageal vagal afferents has yet to be determined.

To study esophageal vagal sensory afferent functions, we have developed an ex vivo guinea pig esophageal-vagal preparation for extracellular single-unit recording. Using this preparation, we have defined that vagal afferent nerve fibers in the guinea pig esophagus are derived from both nodose and jugular ganglia, which include nodose C/Aβ fibers and jugular C/Aδ fibers. Each subtype of these afferents distinctively responds to mechanical distension and chemical stimulation by P2X and TRPVI-receptor agonists (23). We also demonstrated that bradykinin perfusion (22) and mast cell activation (21) displayed different activation and/or sensitization effects on esophageal vagal afferent subtypes.

In the present study, we test the hypothesis that cationic protein has different activation and/or sensitization effects on esophageal vagal afferent subtypes. To perform the study in our ex vivo extracellular recording system, we need to apply fairly large quantities of cationic proteins into afferent nerve endings in the perfusion chamber. This requires purifying a large amount of proteins from a large number of animals. Previous studies have shown that synthetic cationic protein has similar sensitization effects as eosinophil cationic protein and major basic protein on vagal C fibers in the airway (6, 13, 14). Thus we selected synthetic cationic protein to study their effects on esophageal vagal afferent subtypes. Our data demonstrated that synthetic cationic protein poly-L-lysine (PLL) selectively sensitizes esophageal nodose C and Aδ fibers and changes their excitabilities in response to esophageal distension. These results add new evidence that synthetic cationic protein plays a crucial role in regulation of esophageal vagal sensory afferent functions.
MATERIALS AND METHODS

Male Hartley guinea pigs (Hilltop Laboratory Animals, Scottsdale, PA) weighing 100–300 g were used. All experiments were approved by the Penn State Animal Care and Use Committee.

Extracellular single unit recording from vagal node or jugular ganglia neuron with receptive field in the esophagus using ex vivo esophageal-vagal preparation. The esophageal-vagal preparation was set up and extracellular single-unit recordings were performed as previously described (22, 23). The esophagus and trachea were dissected with intact bilateral extrinsic vagal innervation (including jugular and nodose ganglia). The tissue was pinned in a small Sylgard-lined Perspex chamber filled with Krebs’ solution (35°C) [KBS, composed of (in mM) 118 NaCl, 5.4 KCl, 1.0 NaH2PO4, 1.2 MgSO4, 1.9 CaCl2, 25.0 NaHCO3, and 11.1 dextrose, and gassed with 95% O2-5% CO2]. The chamber had two compartments: the esophagus with attached trachea (to support the recurrent laryngeal nerves) and the vagus were pinned in the tissue compartment, and the rostral aspect of the vagus nerves including the nodose and jugular ganglia were pinned in the recording compartment. The two compartments were separated by a silicone grease plug and were separately superfused with KBS (pH 7.4; 35°C; 4–6 ml/min).

Polyethylene tubing was inserted 3–5 mm into the cranial and caudal esophagus and secured for perfusion. The pressure in the fluid (KBS)-filled esophagus was measured with a differential pressure transducer connected in series to the esophagus and recorded simultaneously with neural activity by the chart recorder (TA240S, Gould, Valley View, OH). Isobaric esophageal distension for 20 s with an intraluminal pressure of 10–100 mmHg separated by at least 60 s was used to determine the distension pressure-nerve activity relationship of an esophageal afferent fiber. Distension with a pressure of 10 or 60 mmHg (20 s) was routinely used to assess the viability and mechanical responsiveness of an afferent fiber during experiments.

Extracellular recordings were performed by using an alumino-silicate glass microelectrode (pulled with a Flaming-Brown micropipette puller, Sutter Instrument, Novato, CA) filled with 3 M sodium chloride (electrode resistance 2 MΩ). The microelectrode was placed into either a nodose or jugular ganglion with an electrode holder connected directly to the headstage (A-M Systems, Everett, WA). A return electrode of silver-silver chloride wire and earthed silver-silver chloride pellet was placed in the perfusion fluid of the recording compartment. The recorded signal was amplified (Microelectrode AC Amplifier 1800, A-M Systems) and filtered (low cutoff, 0.3 kHz; high cutoff, 1 kHz), and the resultant activity was displayed on an oscilloscope (TDS 340, Tektronix, Beaverton, OR) and chart recorder. The data were stored and analyzed on a Macintosh computer using the software TheNerveOfIt (sampling frequency 33 kHz; PHOCIS, Baltimore, MD).

To identify a vagal afferent nerve fiber projecting to the esophagus, a distension-sensitive unit was identified when esophageal distension (with a rapid increase in intraluminal pressure to 60 mmHg for 5 s) evoked an action potential discharge. The serosal surface of the esophagus was then searched with a punctate mechanical probe (Von Frey hair, 1 mN, filament diameter < 0.5 mm) applied to the tissue, with a firm probe (outside diameter ~1 mm) having already been inserted into the esophageal lumen. A mechanosensitive receptive field was located when the punctate stimulus evoked a discharge of action potentials. Two waveforms of identical action potentials evoked by distension. The receptive field was then stimulated electrically (pulse duration = 1 ms, frequency = 1 Hz) by use of a concentric electrode inserted into the esophagus with the tip positioned at the site of the mechanosensitive receptive field. The initial voltage (100 V) was gradually reduced to the lowest voltage (threshold voltage) at which each stimulation pulse was followed by a single action potential (30–90 V for most afferent nerve fibers recorded). The waveforms of the electrically evoked action potentials were identical to those induced by distension and the punctate mechanical stimulus. Conduction time was measured as the time between the stimulation pulse and the action potential (visualized by oscilloscope). Variability of conduction time (during the train stimulation, 10 Hz, 10 s) of less than 3 ms indicates direct electrical stimulation (indirect activation of a unit by electrically evoked muscle contractions is readily discernable by high variability > 20 ms of conduction time). Conduction velocity was then calculated by dividing the length of the approximated nerve pathway (from the recorded nodose/jugular neurons to the mechanosensitive receptive field) by conduction time. The nerve fiber was considered a C fiber if it conducts action potentials at < 1 m/s.

The chemicals diluted in KBS solution were delivered to the esophagus in the external perfusate for 10–30 min in the tissue compartment. The nerve activity (action potential discharge) was monitored continuously and analyzed in 1-s bins (yielding the number of action potentials in each second, Hz). The response to the particular stimulus was considered positive when the stimulus evoked action potential discharges with a peak frequency of at least 3 Hz (in the fibers with no baseline activity) or a peak frequency at least three times the frequency of baseline activity. The time to onset of the response was defined as the time elapsed between adding the drug to the tissue to the onset of the action potential discharge. The compounds used in the experiment include: poly-L-lysine, PLGA, α,β-methylene-ATP, AITC, and capsaicin (all from Sigma-Aldrich, St. Louis, MO). The stock solutions of poly-L-lysine (10 mM), poly-L-glutamic acid (10 mM), capsaicin (10 mM), and α,β-methylene-ATP (10 mM) were stored at −20°C and diluted in KBS to final concentration on the day of use. AITC (95%) was diluted with KBS into the final concentration (1 mM) on the day of use.

According to previous studies (6, 14), we selected PLL with molecular weight (MW) of 70,000–150,000 and PLGA with MW of 50,000–100,000. This makes the molar ratio of PLGA/PLL around 1, which may suggest a direct neutralization of positive charges of PLL by PLGA. The final concentration of PLL used in the study was 50 μg/ml in KBS. We selected this higher concentration of the compound on the basis of our pilot experiment (data not shown) and two considerations. First, increased infiltration of eosinophils was identified not only in the mucosa but also in the myenteric muscle layer of the esophagus in patients with EoE (20) and achalasia (12). Upon degranulation, the local concentration of cationic proteins could be higher, which might influence the function of sensory nerve endings in the proximity. Second, in our ex vivo esophageal-vagal preparation PLL was delivered into perfusion compartment of the recording chamber where the recorded nerve ending is not directly exposed to the drug. This is especially meaningful when considering that esophageal vagal Aδ fiber terminals are located in myenteric ganglia which are distant from the mucosa and serosa.

Experimental protocol. In one series of studies, the responses of esophageal vagal afferents to poly-L-lysine were characterized in consecutively recorded nodose C fibers (n = 20), jugular C fibers (n = 10), and nodose Aδ fibers (n = 8). Whether PLL perfusion evoked action potential discharges was first determined. Then mechanocexcitability of these vagal afferents to esophageal distension at pressures of 10, 30, and 60 mmHg were compared before and after PLL perfusion for 30 min.

In another series of studies, nodose C/Aδ fibers were selected to study the mechanism of PLL-induced sensitization of mechanoeexcitability. The effects of poly-L-glutamic acid, which neutralized positive charges of PLL, on PLL-induced changes in mechanoeexcitability were studied in nodose C fibers (n = 10) and Aδ fibers (n = 8).

Data analysis. The distension-evoked nerve response was quantified as the peak frequency of the action potential discharge within a 20-s distention period. Chemical perfusion-evoked response was quantified as the peak frequency of action potential discharge within 10–30 min from the start of the response after the spontaneous activity (if present) was subtracted. The peak frequencies (Hz) of the action potential discharges were presented as means ± SE and
Table 1. Effects of synthetic cationic protein PLL on esophageal vagal afferent subtypes

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<th>Chemoexcitability</th>
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<td>TRPA1</td>
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<td>TRPV1</td>
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<td>In TRPA-TRPV1+ fibers</td>
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PLL, poly-l-lysine; PLGA, poly-l-glutamic acid.

The average conduction velocity of nodose C fibers was 0.59 ± 0.05 m/s (n = 30), that of jugular C fibers was 0.6 ± 0.04 m/s (n = 10), and that of nodose Aδ fibers was 2.8 ± 0.2 m/s (n = 16). The responses of vagal sensory afferent subtypes to mechanical distension and chemical perfusion are detailed below and summarized in Table 1.

Synthetic cationic protein PLL did not activate but did sensitize esophageal nodose C fibers to increase their mechanoexcitability. Extracellular recordings in guinea pig esophageal-vagal preparations were performed to determine the activation effect of PLL on esophageal nodose C fibers. Esophageal distension-evoked action potentials were also compared before and after PLL perfusion. Our data shown that PLL perfusion (50 μg/0.1 ml in KBS, 30 min) in esophageal nodose C fibers (20/20) did not evoke action potential discharges but increased their mechanoexcitability by about twofold (Fig. 1, A and B). The increased action potential discharges evoked by esophageal distension were sustained after washing PLL out for 30 min with fresh buffer (Fig. 1C). Only nodose C fibers that were confirmed to respond to either TRPA1 agonist AITC (1 mM, n = 18) or TRPV1 agonist capsaicin (1 μM, n = 2) at the end of each study were included in the analysis.

Pretreatment with PLGA, which neutralized positive charges of PLL, prevented PLL-induced sensitization of esophageal nodose C fibers. Recent studies suggested that sensitization effect of PLL on nodose C fiber in the airway results from its cationic charges, and pretreatment with PLGA can neutralize positive charges of PLL and prevent such sensitization effect (6, 14). In the present study, we studied the prevention effect of PLGA on PLL-induced sensitization of esophageal nodose C fibers. Perfusion with PLGA itself (3 μM, 30 min) did not evoke action potential discharges (data not shown). Also, action potentials evoked by esophageal distension were unchanged before and after PLGA perfusion (control: 3.1 ± 0.9, 7.3 ± 1.7, and 11.7 ± 2.0 Hz vs. PLGA: 3.6 ± 0.9, 7.6 ± 1.3, and 11.8 ± 1.9 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively, P > 0.05, n = 10). After perfusion with and continually in the presence of PLGA, PLL perfusion (50 μg/0.1 ml in KBS, 30 min) did not increase the mechanoexcitability of these nodose C fibers (Fig. 2). Only nodose C fibers that were confirmed to respond to TRPA1 agonist AITC (1 mM, n = 10) at the end of each study were included in the analysis. These data suggested that neutralizing positive charges of PLL by PLGA prevented its sensitization effect on the mechanoexcitability of esophageal nodose C fibers.

Fig. 1. Nodose C fiber in response to synthetic cationic protein poly-l-lysine (PLL). A: peaks of action potential discharges evoked by esophageal distensions significantly increased from 2.75 ± 0.61, 6.15 ± 1.07, and 11.2 ± 1.31 Hz (control) to 4.8 ± 1.35, 13.2 ± 1.67, and 19.5 ± 1.91 Hz (after PLL) at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively (***P < 0.05, n = 20). B: typical trace of nodose C fiber’s responses (action potentials) to esophageal distension before (control) and after PLL perfusion. C: after washout of PLL for 30 min, the peaks of action potential discharges evoked by esophageal distensions remained significantly greater than control at 5.3 ± 1.1, 9.4 ± 1.4, and 17.4 ± 1.8 Hz (compared with control of 2.3 ± 0.3, 4.8 ± 0.4, and 8.8 ± 0.9 Hz, at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively, **P < 0.05, n = 10).
In esophageal nodose Aδ fibers, synthetic cationic protein PLL did not activate but decreased both the spontaneous activity and mechanoexcitability. In esophageal nodose Aδ fibers (n = 8), PLL perfusion itself (50 μg/0.1 ml in KBS, 30 min) in esophageal-vagal preparations did not evoke action potential discharges but decreased both the spontaneous activity (showing at distension pressure at 0 mmHg) and the excitability in response to esophageal distension (Fig. 3, A and B). To determine whether such decreased excitability sustained in the absence of the PLL, we washed PLL out for 30–60 min with fresh buffer in four of the eight nodose Aδ fibers. We observed that both decreased spontaneous activity and mechanoexcitability was restored after washing PLL out for 30–60 min (Fig. 3C). Only nodose Aδ fibers that were confirmed to respond to P2X agonist α,β-methylene-ATP (30 μM, n = 8) at the end of each recording were included in the analysis.

### Pretreatment with PLGA, which neutralized positive charges of PLL, prevented PLL-induced decreases in spontaneous activity and mechanoexcitability of esophageal nodose Aδ fibers.

Similar to the observation on esophageal nodose C fibers, perfusion with PLGA (3 μM, 30 min) itself did not evoke action potential discharges in esophageal nodose Aδ fibers (control vs. after-PLGA: 5.0 ± 0.9 Hz vs. 5.0 ± 1.2 Hz, P > 0.05, n = 8). Esophageal distension-evoked action potential discharges were unchanged before and after PLGA perfusion (20.4 ± 3.0, 27.7 ± 2.2, and 28.9 ± 3.0 Hz vs. PLGA+PLLA: 16.7 ± 0.9, 22.6 ± 2.6, and 23.3 ± 2.8 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively, P > 0.05, except at pressure of 60 mmHg, n = 8).

### Responsive Nodose Aδ fibers

- **A**: in nodose Aδ fibers, PLL perfusion (50 μg/0.1 ml in Krebs’ solution, 30 min) did not evoke action potential discharges, but the spontaneous action potential discharges (showing at distension pressure of 0 mmHg) decreased from 3.1 ± 0.9, 7.3 ± 1.7, and 11.7 ± 2.0 Hz vs. PLGA+PLL: 3.8 ± 0.9, 7.8 ± 1.6, and 12.5 ± 2.0 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively, (P < 0.05, n = 8).

- **B**: typical trace of nodose Aδ fiber’s response (action potentials) to esophageal distension before and after PLL perfusion.

- **C**: after PLL was washed out for 30–60 min, the peaks of action potential discharges evoked by esophageal dissections were restored to 20.3 ± 1.8, 27.5 ± 2.6, and 33.3 ± 4.5 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively (vs. control, P > 0.05, n = 4).

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**Fig. 2.** Nodose C fiber in response to synthetic cationic protein PLL after pretreatment with poly-l-glutamic acid (PLGA). Compared with control, PLL perfusion did not significantly change the action potential discharges evoked by esophageal distension after perfusion with and continually in the presence of PLGA (control: 3.1 ± 0.9, 7.3 ± 1.7, and 11.7 ± 2.0 Hz vs. PLGA+PLL: 3.8 ± 0.9, 7.8 ± 1.6, and 12.5 ± 2.0 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively, P > 0.05, n = 10).

**Fig. 3.** Nodose Aδ fiber in response to synthetic cationic protein PLL. A: in nodose Aδ fibers, PLL perfusion (50 μg/0.1 ml in Krebs’ solution, 30 min) did not evoke action potential discharges, but the spontaneous action potential discharges (showing at distension pressure of 0 mmHg) decreased from 3.1 ± 0.9, 7.3 ± 1.7, and 11.7 ± 2.0 Hz vs. PLGA+PLL: 3.8 ± 0.9, 7.8 ± 1.6, and 12.5 ± 2.0 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively, (P < 0.05, n = 8). B: typical trace of nodose Aδ fiber’s response (action potentials) to esophageal distension before and after PLL perfusion. C: after PLL was washed out for 30–60 min, the peaks of action potential discharges evoked by esophageal dissections were restored to 20.3 ± 1.8, 27.5 ± 2.6, and 33.3 ± 4.5 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively (vs. control, P > 0.05, n = 4).
end of each recording were included in the analysis. This result suggested that neutralizing positive charges of PLL by PLGA attenuated the PLL-induced sensitization effect on the mechanoexcitability of esophageal node A6 fibers.

Synthetic cationic protein neither activated nor changed the mechanoexcitability of esophageal jugular C fibers. In esophageal jugular fibers (n = 10), PLL perfusion (50 μg/0.1 ml in KBS, 30 min) in esophageal-vagal preparations did not evoke any action potential discharges. The mechanoexcitability of esophageal nodose C fiber in response to esophageal distension did not change after PLL perfusion (Fig. 5, A and B). All studied jugular fibers were confirmed to respond to TRPA1 agonist AITC (action potential discharges at 11.1 ± 1.5 Hz over the baseline), which is used as a tool to confirm that the recorded nerve terminals are exposed to drug perfusion.

DISCUSSION

Normal human esophageal tissue only has minimal eosinophils (9). But this can be changed in inflamed esophagus (2). Significantly increased infiltration and degranulation of eosinophils are the predominant features of EoE (16). In esophageal biopsy, 15 eosinophils per high power field under microscope is considered a minimum threshold for a diagnosis of EoE (15). Such a large number of eosinophils, upon activation, can release high concentration of granule proteins into the esophageal tissue, which may regulate sensory afferent functions in the proximity. In the present study, we applied synthetic cationic protein to determine their effects on esophageal afferent function. Our data demonstrated that synthetic cationic protein has different effects on esophageal vagal afferent nerve subtypes. In esophageal nodose C fiber, perfusion with PLL does not evoke action potential discharges but does sensitize their response to esophageal distension. In esophageal nodose A6 fiber, perfusion with PLL inhibits both spontaneous discharges and their response to esophageal distension. These effects can be prevented by pretreatment with PLGA. In contrast, perfusion with synthetic cationic protein PLL neither evokes action potential discharges nor sensitizes their response to esophageal distension in esophageal jugular C fiber.

A series of studies using single-fiber recording in vivo has shown that intratracheal instillation with either eosinophil-derived cationic protein (13) or synthetic cationic protein (6) directly activated vagal pulmonary C fibers and increased their response to both lung inflation and chemical stimuli. Further studies from the same group using patch-clamp recording on vagal pulmonary neurons clearly demonstrated that eosinophil-derived cationic protein did not directly activate those dissociated neurons but sensitized their response to other chemical stimuli such as acid, ATP, and capsaicin. All of these effects could be abolished by pretreatment with polyanions, which neutralized the cationic charges of those cationic proteins.

The present study provides the new evidence for an effect of synthetic cationic protein on esophageal vagal afferent subtypes. The observation that PLL failed to evoke action potential discharges in esophageal nodose C fibers but substantially increased their excitability to esophageal distension is similar to the results obtained from vagal pulmonary neurons. The lack of overtly activation of esophageal nodose C fibers by PLL in the present study is different from aforementioned in vivo effect of PLL on vagal pulmonary C fibers. Another particular interesting difference was that PLL didn’t show any effect on esophageal jugular C fibers in the present study compared with the previous study, which demonstrated a sensitization effect on pulmonary jugular neurons. We thought that several factors might contribute to these differences. First, we carried out our single-fiber recording ex vivo and the recording electrode was placed into nodose neuron. This minimized the background activity. Second, the neural crest-derived jugular C fibers and the placodal-derived nodose C fibers respond to mechano- and chemostimuli differently as shown in the esophagus (23) and the lung (17). Third, there may be differences between esophageal and pulmonary vagal C fibers. In addition, different species were used in the two studies.

The underlying mechanism of synthetic cationic protein-induced sensitization of esophageal vagal afferent C fiber is less clear. Previous study in vagal pulmonary neuron demonstrated that inhibition of voltage-gated K+ current and A-type fast-inactivating K+ current, but not interaction with TRP V1 channels, may play a role in this sensitization process (7). In the present study, we observed that PLL sensitized either TRPA1+ or TRPA−/TRPV1+ nodose C fibers but had no effect on TRPA1+ jugular C fibers. We speculate that different sensitization effects of synthetic cationic protein on esophageal nodose and jugular C fibers may be resulted from sensitization of ion channels or receptors that are distinctively expressed on

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**Fig. 5. Jugular C fiber in response to synthetic cationic protein PLL.** A: peaks of action potential discharges evoked by esophageal distensions did not change before and after PLL perfusion (1.4 ± 0.2, 3.0 ± 0.5, and 6.2 ± 0.8 vs. 1.6 ± 0.3, 3.3 ± 0.6, and 5.7 ± 0.9 Hz at distension pressures of 10, 30, and 60 mmHg for 20 s, respectively, P > 0.05, n = 10). B: typical trace of jugular C fiber’s responses (action potentials) to esophageal distension before and after PLL perfusion.
those subtype afferents. Our previous studies have demonstrated that TRPA1 plays a crucial role in bradykinin and mast cell activation-induced sensitization of esophageal vagal C fibers (21, 22). A conclusive role of TRPA1 in P2X-induced sensitization of esophageal vagal C fibers needs to be further clarified. Our previous study demonstrated that there is a subpopulation of vagal afferents, Aδ fibers with neuron cell bodies in nodose ganglion, also innervated in the esophagus in addition to nodose C fiber. They are characterized to have fast conduction velocity, display saturated response to esophageal distension at noxious range (more than 30 mmHg), and can be activated by P2X receptor agonist α,β-methylene-ATP (23). Currently, to the best of our knowledge, there is no report on the effects of (eosinophil) cationic protein or major basic protein on vagal afferent Aδ fibers. In the present study, perfusion with synthetic cationic protein lead to decreases not only in the spontaneous discharges but also in the mechanoeexcitability of esophageal nodose Aδ fibers. This provides new evidence on esophageal nodose Aδ fiber sensitization. The underlying mechanism of such inhibition effect is unknown. One speculation is that there might be an interaction between cationic protein and P2X receptor. P2X receptor is considered to involve in mechanosensory transduction. Perfusion with P2X receptor agonist α,β-methylene-ATP activated esophageal nodose Aδ fiber. This was usually followed by a short silent period in response to esophageal distension, which may be similar to P2X-induced inhibition on mechanoeexcitability. P2X seems unlikely to activate P2X receptor in nodose Aδ fiber; otherwise we might have observed an activation response. Guinea pig esophageal nodose Aδ fibers are intraganglionic laminar endings that are located in esophageal myenteric ganglia (24). Whether P2X acts on Aδ fiber nerve terminals and further influences the function of myenteric neurons, especially those cholinergic neurons as shown in the airway (5), deserves a further exploration.

Increased infiltration of eosinophils in the esophagus has been observed in patients with noncardiac chest pain (1). Esophageal distensibility was significantly reduced in EoE patients (11). Dysfunction of the esophageal longitudinal muscles was reported in patients with EoE (10). These clinical evidences suggest that increased infiltration and degranulation of eosinophils in the esophagus might contribute to esophageal sensory motor dysfunctions in EoE patients. The present study provides the first evidence that synthetic cationic protein does not directly activate esophageal vagal afferent subtypes but does display distinctive sensitization effects on their mechanoeexcitabilities. This will help to further clarify the roles of eosinophil-derived cationic protein and major basic protein in sensitization of esophageal sensory afferents, which may lead to a better understanding of the mechanism of esophageal sensory motor dysfunctions in EoE.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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