

Modulation of Airway Sensitivity to Inhaled Irritants: Role of Inflammatory Mediators

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Bronchopulmonary C-fiber endings and rapidly adapting pulmonary receptors (RARs) are primarily responsible for eliciting the defense reflexes in protecting the lungs against inhaled irritants. In anesthetized animals, inhalation of cigarette smoke, one of the common inhaled irritants, into the lungs elicits pulmonary chemoreflexes that are mediated through the stimulation of pulmonary C fibers. When the C-fiber conduction is selectively blocked in the vagus nerves, the same smoke inhalation triggered only augmented breaths, a reflex effect of activating RARs, in the same animals. Indeed, electrophysiologic study shows that inhaled smoke exerts a direct stimulatory effect on both types of afferents. Increasing evidence indicates that the excitability of these afferents and therefore their reflex actions are enhanced by airway mucosal inflammation; one such example is the airway hyperresponsiveness induced by acute exposure to ozone. Although the mechanism underlying the inflammation-induced hypersensitivity of C-fiber endings is not fully understood, the possible involvement of local release of certain inflammatory mediators, such as histamine and prostaglandin E₂ (PGE₂), should be considered. It is believed that changes in the membrane properties mediated by the activation of certain specific receptor proteins located on the membrane of these nerve terminals are involved, as the sensitizing effects of PGE₂ can be also demonstrated in cultured pulmonary C neurons. *Key words:* airway inflammation, airway receptors, airway reflexes, bronchial hyperreactivity, cigarette smoke, inhaled irritants, ozone. — *Environ Health Perspect* 109(suppl 4):585–589 (2001). <http://ehpnet1.niehs.nih.gov/docs/2001/suppl-4/585-589lee/abstract.html>

The important function of the airway reflexes in protecting the lungs against inhaled irritants is well recognized and extensively documented (1–3). The majority of these reflex responses are elicited by activation of sensory receptors located in the airways and lungs. Afferent activities arising from these receptors are conducted almost exclusively in the vagus nerves. In this short review, we focus on several recent studies carried out in our laboratories to identify the type(s) of vagal bronchopulmonary receptors involved in eliciting these defense reflexes and the changes of sensitivities of these receptors caused by airway mucosal inflammation.

Airway Afferents Involved in the Reflex Responses to Inhaled Irritants

Among the three major types of sensory receptors innervating the airways and lungs, C-fiber endings and rapidly adapting pulmonary receptors (RARs or irritant receptors) are believed to be primarily responsible for eliciting the reflex responses in defending the lungs against inhaled irritants and toxins (1–3). The third type, slowly adapting pulmonary receptors (SARs or stretch receptors), plays an important role in regulating the respiratory volume-timing relationship (4), and is considered to be pure mechanoreceptor and relatively insensitive to chemical irritants. Several recent studies have attempted to uncover the mechanisms underlying the interaction

between the pulmonary C fibers and RARs in the overall regulation of the defense reflex responses. Inhalation of cigarette smoke, a common inhaled irritant in human airways, has been shown to elicit irregular breathing pattern, cough reflex, and bronchoconstriction in a number of species including humans (5–9). Although it is believed that most of these responses are mediated through the activation of these two types of vagal afferents, the relative contributions of these two types of afferents to the emergence of these reflex responses are not known. In anesthetized animals (dogs, cats, or rats), spontaneous inhalation of one to two tidal breaths of cigarette smoke into the lungs via a tracheal cannula immediately elicited pulmonary chemoreflexes, characterized by apnea, bradycardia, and hypotension, known to be elicited by activation of pulmonary C fibers (1–3,6). After perineural capsaicin treatment of both cervical vagi to selectively block the conduction of capsaicin-sensitive C fibers, inhaled cigarette smoke no longer evoked any inhibitory effect on breathing (9). Conversely, an augmented inspiration, a reflex effect of activating RARs, was triggered within the first three breaths from the onset of cigarette smoke inhalation in > 85% of the rats studied (Figure 1). When the temperature of the vagus nerve was cooled progressively to 6–7 °C to block the conduction of myelinated afferents, as indicated by the ablation of the apneic response to lung inflation, the augmented breaths evoked by

the cigarette smoke inhalation were also abolished (9). Both the apnea and augmented breath evoked by inhaled cigarette smoke were completely abolished by bilateral cervical vagotomy. Similarly, augmented breaths were also elicited when other chemical irritants such as sulfur dioxide (SO₂) (9), ammonia (NH₃) (9), acrolein (10), and wood smoke (11) were inhaled after the perineural capsaicin treatment of both vagi in anesthetized rats.

It is interesting to note that augmented breaths were consistently elicited by inhaled irritants only after the conduction in bronchopulmonary C-fiber afferents are blocked by perineural capsaicin treatment of both vagi but were rarely seen when the same irritants were inhaled in the same animals with intact C fibers (9–11). These results suggest that both vagal bronchopulmonary C-fiber afferents and RARs are stimulated by these inhaled irritants, probably to a varying degree, and the immediate ventilatory changes reflect the integrated reflex response resulting from the activation of these two types of afferents. In intact animals, the inspiratory-excitatory reflex response evoked by the stimulation of RARs was probably overridden by the concomitant but more dominant inhibitory effect mediated by C-fiber afferent activation. Thus, the augmented inspirations elicited by these irritants became more pronounced or unmasked only after the conduction in C fibers had been selectively blocked. Another example demonstrating the central interaction of these two types of vagal afferents and the dominant influence of the inhibitory effect of C-fiber activation is shown in anesthetized cats (12). Mechanical stimulation of the mucosa of the tracheobronchial tree triggered vigorous coughs that sustained even after the termination of the stimulation and were presumably elicited by stimulation of RARs (1).

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However, the same mechanical stimulation failed to elicit the cough response during the stimulation of pulmonary C-fiber afferents induced by right atrial injection of capsaicin or phenylbiguanide (Figure 2).

Because of the irritant nature of cigarette smoke and other inhaled noxious agents, the reflex-mediated responses of breathing to these inhaled irritants in awake human subjects are often distorted by the behavioral influence (3,7). Therefore, accurate data interpretation is more difficult. In addition, because most of the experimental procedures required in these studies cannot be applied to human volunteers, studies of the airway afferents involved in the reflex responses to inhaled irritants have been performed almost exclusively in anesthetized-animal preparations. However, the influence of anesthesia on the regulation of these reflex responses can not be overlooked. For example, in awake dogs inhalation of cigarette smoke via tracheostomy tube immediately evoked augmented breaths in approximately 30% of the animals tested (6). In contrast, when the same smoke inhalation challenge was applied to the same animals under anesthesia, pulmonary chemoreflexes (apnea, bradycardia, and hypotension) were the only responses found and augmented breaths were almost never observed (5,6). This discrepancy suggests that suppression of the central nervous system by anesthetics may have affected the interaction of these afferents and depressed the reflex response mediated through the activation of RARs to a larger degree.

The contention that C-fiber afferents and RARs are primarily responsible for eliciting the reflex responses to inhaled cigarette smoke

is in general agreement with the results obtained from direct recording of the afferent responses of these receptors. For example, cigarette smoke delivered into the lungs of anesthetized, open-chest dogs in a single ventilator cycle immediately evoked a short but intense burst of discharge of pulmonary C fibers (Figure 3). This response was found in approximately 90% of the fibers tested, and was independent of the bronchoconstrictive effect of the smoke. Furthermore, nicotine contained in the cigarette smoke is responsible for generating this stimulatory effect because it can be completely blocked by pretreatment with hexamethonium, the acetylcholine nicotinic receptor antagonist (13). On the other hand, Sellick and Widdicombe (14) reported the first evidence that cigarette smoke stimulates RARs in rabbit lungs. On the basis of their observations, it was suggested that the stimulation of RARs may be involved in the smoke-induced reflex bronchoconstriction. In a more extensive study, Kou and Lee (15,16) later confirmed this finding in dogs but further demonstrated a biphasic response of these receptors to inhalation of a single breath of cigarette smoke (Figure 4). The initial response of RARs occurred immediately after the delivery of smoke into the airways and was related to the nicotine content in the smoke in a manner similar to that observed in the response of C fibers. The delayed response developed 10–20 sec after the smoke inhalation and was caused by nicotine-induced bronchoconstriction, mediated through the cholinergic reflex (16). Taken together, nicotine contained in the cigarette smoke exerts a direct stimulatory effect on both C-fiber afferents and RARs in

the lungs (13–16); the effect is more pronounced and consistent on the former. The electrophysiologic evidence further suggests the presence of acetylcholine nicotinic receptors on the membrane of sensory terminals of these lung afferents (13,16). The finding that nicotine is responsible for the smoke-evoked irritant effects on airway afferents was later confirmed in a study on healthy human volunteers when the intensity and detection of the airway irritation were measured using a psychophysical technique (7).

Excitability of Airway Afferents Modulated by Inflammatory Mediators

Increasing evidence indicates that the excitability of these bronchopulmonary afferents is significantly enhanced when airway inflammation is induced by certain experimental procedures such as acute exposure to ozone (17,18) or sensitization with allergens (19). Considering the important role of these afferents in the pulmonary defense against inhalation assaults in healthy individuals, the enhanced sensitivity developed during the airway inflammation is, presumably, even more critical when additional protection is needed under these disease conditions.

Acute exposure to ozone (O₃), one of the major air pollutants in urban areas, induces mucosal inflammation and transient bronchial hyperreactivity in a number of

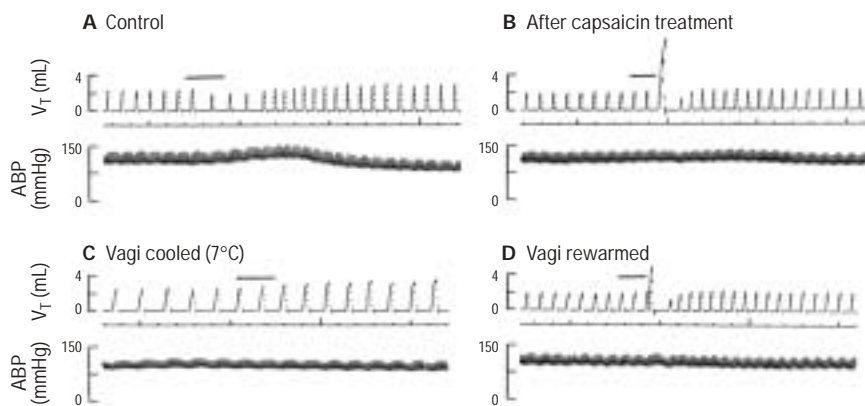


Figure 1. Experimental records illustrating acute responses to cigarette smoke inhalation in an anesthetized rat. V_T , tidal volume; ABP, arterial blood pressure. Horizontal bars were added to depict the time of cigarette smoke (50%, 6 mL) inhalations. (A) Control responses; (B) 10 min after termination of perineural capsaicin treatment of both vagi; (C) 20 min later when reflex apneic response to lung inflation was abolished by cooling both vagi to 7°C; (D), 20 min later when both vagi were rewarmed to 37°C and the apneic response to lung inflation were recovered (not shown). Perineural capsaicin treatment selectively blocked C-fiber conduction (indicated by ablation of reflex responses to capsaicin injection; data not shown) for > 90 min. The mild, delayed hyperpnea that occurred after smoke inhalation in each panel was probably caused by stimulation of peripheral chemoreceptors by the absorbed nicotine (4). Time between two smaller time marks was 1 sec. Data from Wang et al. (9).

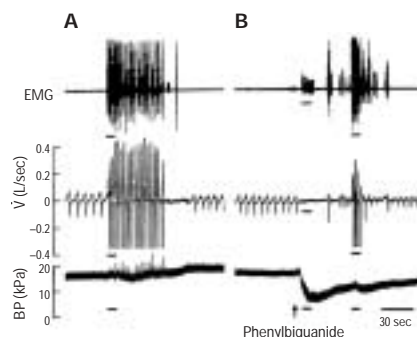


Figure 2. Effect of pulmonary C-fiber reflex on coughing induced from the tracheobronchial tree in an anesthetized cat. Traces from top to bottom: EMG of genioglossus muscle, airflow (V) from tracheal cannula, and systemic arterial blood pressure (BP). (A) The tracheobronchial mucosa was stimulated mechanically during the signal markers, causing increased EMG activity and airflow, corresponding to cough efforts. The cough efforts continued long after the stimulus had stopped. (B) Phenylbiguanide (25 µg/kg) was injected iv at the arrow, causing hypotension, bradycardia, and apnea. During the apnea at the signal markers the tracheobronchial stimulus was repeated, causing no change in airflow but some increase in EMG activity. Later, during the phase of rapid shallow breathing, the tracheobronchial stimulus was repeated and caused four cough efforts, with no coughing after the end of the stimulus. Data from Tatar et al. (12).

species including humans (20,21). Reflex bronchoconstriction mediated through cholinergic pathway is probably involved because the enhanced airway responsiveness to histamine in human subjects after exposure to O₃ (0.6 ppm for 2 hr) was partially eliminated by pretreatment with atropine (20). Furthermore, airway irritation and substernal pain were reported in healthy human subjects shortly after exposure to low concentrations of O₃ (0.5–0.6 ppm for 2 hr); many individuals coughed upon taking a deep inspiration, suggesting an increased excitability of pulmonary afferents (20,22). Indeed, this hypothesis is supported by data obtained from direct recording of pulmonary C fibers. After acute exposure to O₃ (3 ppm for 0.5 hr), there was no significant change in arterial blood pressure, tracheal pressure, or baseline activity of C fibers in anesthetized, open-chest rats. However, the stimulatory effect of the same dose of capsaicin on these fibers was markedly enhanced ($\Delta > 680\%$) immediately after O₃ exposure (Figure 5), and the effect was reversible after approximately 1 hr. Similarly, the pulmonary C-fiber response to injection of a low dose of lactic acid was also elevated after O₃ exposure (Figure 5). Thus, a given level of inhalation challenge with bronchoactive substances may evoke a greater discharge of C-fiber endings, and consequently a more severe bronchoconstriction mediated through both the cholinergic reflex pathway and tachykinin release (8). Furthermore, O₃ exposure significantly potentiated the C-fiber response to hyperinflation of the lungs (Figure 5). This observation is particularly interesting and physiologically relevant because expansion of the lungs is a natural stimulus and occurs in normal physiologic conditions (e.g., hyperventilation during heavy exercise). Although the concentration of O₃ (3 ppm) applied in this study was several times higher than the range of environmental concentrations, the duration of exposure (30 min) was considerably shorter than those employed in other ozone studies; the duration was shortened to successfully maintain the continuous recording of the single-unit C-fiber activities. Joad and co-workers (18) have also reported that exposure to lower concentration of ozone (0.5 ppm for 8 hr/day) for a week increases the excitability of RARs to substance P, methacholine, and lung inflation. The increased responses of RARs were not associated with exaggerated changes in lung mechanics, suggesting a direct action of ozone on these receptors. Taken together, these results suggest that sensitization of these lung afferents is probably involved in evoking the substernal pain, airway irritation, coughing, and airway hyperreactivity in humans following the ozone exposure (17,20,22).

The possible mechanisms underlying the enhanced excitability of these sensory endings

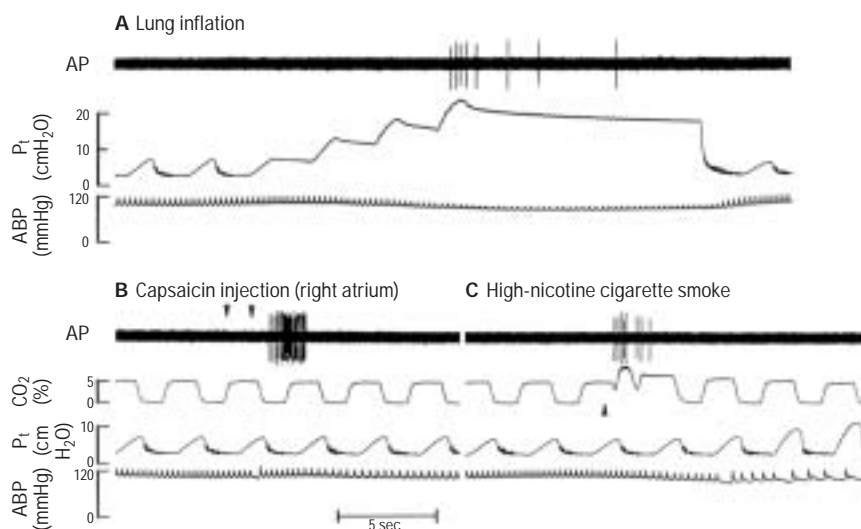


Figure 3. Effects of lung inflation, capsaicin, and cigarette smoke on a pulmonary C-fiber arising from ending in right apical lobe of an anesthetized dog. AP, action potentials; CO₂, CO₂ concentration in trachea; P_i, tracheal pressure; ABP, arterial blood pressure. (A) C-fiber stimulated by hyperinflation of the lungs. (B) Capsaicin (5 μg/kg) injected into catheter at first arrow and flushed into right atrium at second. (C) 120 mL high-nicotine cigarette smoke delivered to lungs in a single ventilatory cycle; note sudden increase in inspiratory carbon dioxide (CO₂) concentration. The arrow whose position has been adjusted to correct for time lag of CO₂ analyzer, indicates arrival of smoke in airways. Interval of 20 min elapsed between injection of capsaicin and inhalation of smoke. Data from Lee et al. (13).

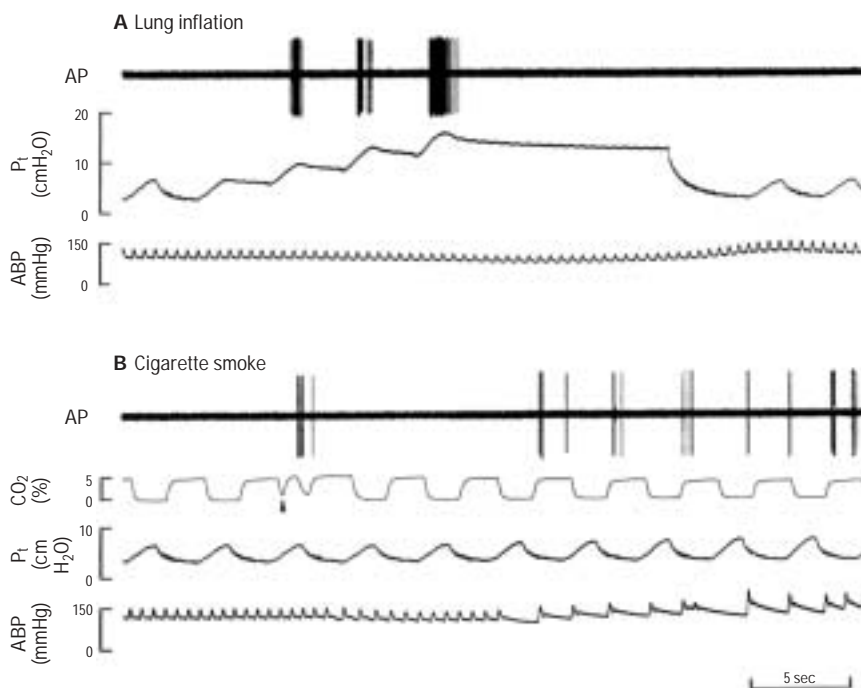


Figure 4. Stimulation of rapidly adapting receptors by hyperinflation of the lungs (A) and by cigarette smoke (B) in an anesthetized dog. Evoked discharge of receptor adapted rapidly to maintained lung inflation (A). Cigarette smoke evoked an initial and a delayed response in the same receptor (B). The delayed hypertension and bradycardia following the smoke inhalation challenge (B) are believed to result from systemic effects of circulated (absorbed) nicotine on various target cells (e.g., adrenal glands, peripheral chemoreceptors, autonomic ganglia). The receptor was located in lobar bronchus supplying cardiac lobe. For detailed explanation, see legend for Figure 3. Data from Kou and Lee (16).

in the lungs after O₃ exposure are not yet fully understood. It has been suggested that O₃-induced epithelial injury and mucosal inflammation in the airways may play a part (17,23,24). In particular, O₃ exposure has been shown to cause the local release in the airways of certain inflammatory mediators known to enhance the sensitivity of these afferent endings. For example, histamine released in the lungs has been shown to increase substantially immediately following exposure to ozone (25). Histamine is a potent inflammatory mediator secreted locally from mast cells and basophils during various inflammatory and allergic reactions in the airways. Histamine is known to have profound effects on a number of target cells in the lungs, and to cause airway smooth muscle contraction, bronchial and pulmonary arteriolar vasodilation, and

hypersecretion of mucus. Lee and Morton (26) have recently reported that inhalation of a low dose of aerosolized histamine (five breaths, 1% concentration) augments the afferent responses of vagal pulmonary C fibers to both lung inflation and right atrial injection of capsaicin in anesthetized, open-chest dogs. Histamine was delivered into the lungs by inhalation route to minimize its sustaining and diffusive systemic effects. The dose of histamine actually deposited in the lungs was not determined in their study, but it was estimated to be comparable to the higher levels of endogenously released histamine, judging from the degree of bronchoconstriction caused by the inhalation challenge (26). Their results show that this potentiating effect on C-fiber endings is not related to the bronchoconstrictive effect of histamine and is reversible 15–25 min later.

Another group of autacoids that should be considered as potential candidates in sensitizing the pulmonary C-fiber endings after O₃ exposure are certain arachidonic acid metabolites. The airway epithelium, which is the site of initial assault by O₃, is also a primary cellular source of these autacoids in the lung (27).

In fact, the bronchial hyperreactivity induced by O₃ (3 ppm for 2 hr) is prevented by premedication with indomethacin (28), suggesting that local release of cyclooxygenase metabolites resulting from the O₃-induced epithelial injury plays a part in the afferent sensitization in anesthetized dogs. Moreover, elevated levels of several prostanoids, including prostaglandin E₂ (PGE₂), in the lung lavage fluid have already been documented after acute exposure to O₃ (0.4–0.6 ppm for 2 hr) in man (27). PGE₂, a potent inflammatory mediator derived from arachidonic acid metabolism through the enzymatic action of cyclooxygenase and PGE synthase, is generated and released by epithelial cells during various airway inflammatory reactions (27). PGE₂ causes airway and vascular smooth muscle relaxation and modulates the functions of other inflammatory cells (e.g., neutrophils) (29,30). PGE₂ is also known to activate sensory endings in the lung. For example, inhalation of aerosolized PGE₂ elicits coughs and retrosternal soreness without significant change in baseline airway resistance (31,32) and augments the dyspneic sensation during exercise (31) in healthy human subjects. Inhalation of PGE₂ aerosol also induces reflex bronchoconstriction in asthmatic patients, despite its potent direct dilating effect on airway smooth muscles (27). Furthermore, inhaled PGE₂ enhances the sensitivity of the cough reflex elicited by capsaicin in humans (33), suggesting a PGE₂-induced sensitization of pulmonary C-fiber afferents. Indeed, recent study by Ho et al. (34) demonstrated that constant intravenous (iv) infusion of a low dose of PGE₂ (1.5 µg/kg/min for 2 min), which produced only a slight drop in arterial blood pressure ($\Delta = 7$ mmHg), markedly enhanced the stimulatory effects of both low (0.25 µg/kg, iv) and high doses (0.5 µg/kg, iv) of capsaicin on these fibers in anesthetized, open-chest rats (Figure 6). Similarly, potentiating effects of PGE₂ were found on the pulmonary C-fiber responses to injections of lactic acid and adenosine. In addition, PGE₂ infusion also significantly enhanced the C-fiber response to constant-pressure lung inflation (Figure 6). In contrast, the same dose of PGE₂ did not cause any detectable change in the sensitivity of either RARs or slowly adapting pulmonary stretch receptors to lung inflation (34).

Whether the sensitizing effects of these autacoids on C-fiber afferents are caused by a direct action on the sensory endings or by a secondary effect through the action of other cells in the lung cannot be determined. However, Kwong and Lee in a recent study (35) have reported direct evidence in support of the former; when the inward current evoked by capsaicin challenge (10⁻⁶ M) was measured using a whole-cell voltage-clamp

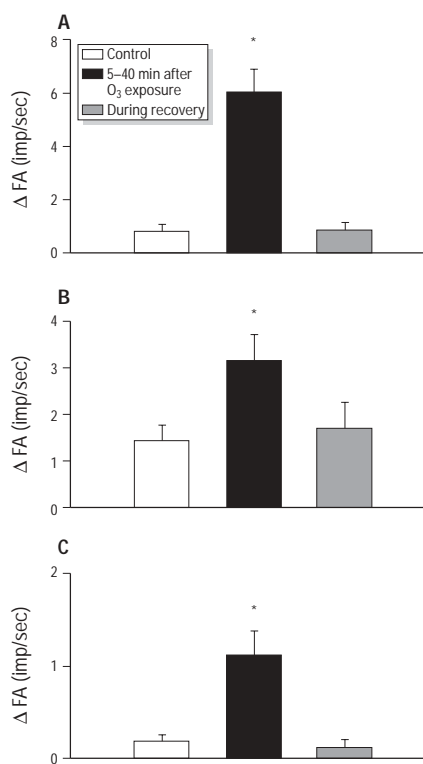


Figure 5. Effects of ozone on pulmonary C-fiber responses to right-atrial injections of capsaicin [0.5–1 µg/kg; (A)], lactic acid [0.1 mmol/kg; (B)], and lung inflation [$P_i = 30$ cmH₂O; (C)] in anesthetized, open-chest rats. FA, fiber activity. Δ FA represents the difference between the peak FA (averaged over 2-sec intervals in the responses to capsaicin and lactic acid injections, and over the 10-sec interval in the response to lung inflation) and the baseline FA (averaged over 10-sec intervals). Open bars, control responses; closed bars, responses 5–40 min after the cessation of O₃ exposure (3 ppm for 30 min); shaded bars, responses during recovery. Numbers of C fibers studied were 31 (A), 30 (B), and 29 (C) during control and after O₃; 26 (A), 8 (B), and 10 (C) during recovery. Data represent means \pm SE. * $p < 0.01$. Data from Ho and Lee (17).

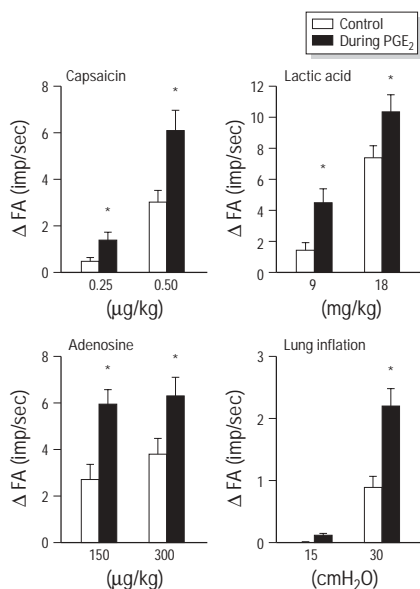


Figure 6. A comparison of average pulmonary C-fiber responses to injections of chemical stimulants and to lung inflation between control and during PGE₂ infusion in anaesthetized, open-chest rats. In the responses to injections of capsaicin ($n = 27$), lactic acid ($n = 24$), or adenosine ($n = 20$), Δ FA represents the difference in FA between the peak response (averaged over 2-sec intervals) and the baseline FA (averaged over 10-sec intervals). In the response to lung inflation ($n = 45$), Δ FA represents the difference between FA during the 10-sec interval and the baseline FA (each averaged over a 10-sec interval). Open bar, control response; closed bar, response during PGE₂ infusion. Data represent means \pm SE. * $p < 0.05$, compared with the control response. Figure modified from Ho et al. (34).

configuration in the cultured rat nodose and jugular C neurons that had been retrogradely labeled with fluorescent dye instilled into the airways, the response was markedly potentiated when the neurons were superfused with PGE₂ (10⁻⁷ to 10⁻⁶ M) (35). Although the cellular mechanism by which these autacoids induce hypersensitivity of pulmonary C-fiber afferent endings is not fully understood, it is believed that changes in the membrane properties mediated by the activation of certain specific receptor proteins located on the membrane of the nerve terminals are involved. Among the several types of prostanoid receptors, the EP receptor has the highest affinity for PGE₂ based upon the ligand-binding studies, and some of the subtypes of the EP receptor, such as EP₂, EP₃ and EP₄ receptors, are known to be present on the sensory nerves and may therefore be involved in mediating the sensitizing effects of PGE₂ on these endings (29,30). Several species of G protein are known to participate in signal transduction via the EP receptors (30). Both EP₂ and EP₄ receptors are coupled to Gs proteins that mediate increases in cyclic AMP (cAMP) level. Recent studies in rat dorsal root ganglion (DRG) nociceptive neurons have shown that PGE₂-induced sensitization is due to an increase of the enzyme activity of adenylyl cyclase and the resulting rise in the level of intracellular cAMP (36). Cyclic AMP can then stimulate protein kinase A (PKA), which in turn enhances the neuronal excitability by increasing the phosphorylation of various ion channels. This hypothesis is supported by the observation made in C neurons in DRG; the hypersensitizing effects of PGE₂ on DRG neurons can be mimicked by forskolin, a direct activator of adenylyl cyclase, and attenuated by inhibiting the PKA activity (36). Taken together, the evidence suggests that EP₂ and EP₄ receptors are probably the prostanoid receptors that mediate the sensitizing action of PGE₂ on the pulmonary C neurons (37). Whether the second-messenger cAMP/PKA signaling pathway is involved in the PGE₂-induced hypersensitivity of these neurons remains to be determined.

Conclusion

Bronchopulmonary C fibers and RARs are primarily responsible for eliciting the airway reflexes which protect the lungs against inhaled irritants. This conclusion is supported by experimental results obtained from direct recording of their afferent activities as well as from the study of the reflex responses to various inhaled irritants. The relative

contributions of these two types of afferents are influenced by a number of factors such as the types of stimulus, the state of wakefulness, etc. The sensitivities of these sensory receptors are enhanced by airway inflammation, which presumably heightens the airway defense functions during the disease conditions. Although the mechanism underlying the enhanced sensitivities of these afferent endings is not yet fully understood, it probably involves local release of certain inflammatory mediators (e.g., prostaglandins, histamine) in the airways. By activating specific receptor proteins (e.g., subtypes of the prostanoid receptor) located on the membrane of the sensory terminals, these autacoids can alter the membrane properties and elevate the excitabilities of these afferent endings. The intracellular signal transduction pathways and specific ionic channels involved remain to be explored.

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