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Review

Pulmonary alterations associated with inhalation of occupational and environmental irritants

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Abstract

Many gases, vapors, or particles found in occupational and/or environmental settings can act as irritants. In the present study, sensory irritants are characterized by the stimulation of neuropeptide release from sensory nerves in the nasal mucosa, while pulmonary irritants are characterized by recruitment of PMN into bronchoalveolar airspaces, elevation of breathing frequency, and neuropeptide release from sensory fibers innervating the epithelium of the conducting airways. A review of data from our laboratory as well as results from others indicate that asphalt fume is a sensory irritant; toluene diisocyanate (TDI), methyl isocyanate, and machining fluid act as both sensory and pulmonary irritants; while cotton dust, agricultural dusts, microbial products, leather conditioner, and ozone exhibit responses characteristic of pulmonary irritants.

Keywords: Sensory irritants; Pulmonary irritants; Neuropeptides; Pulmonary inflammation; Breathing rate; Occupational agents

1. Introduction

Inhaled gases, vapors, or particles found in various occupational or environmental settings can act as irritants to the respiratory system. In a recent review, Alarie et al. [1] classified irritation to the lungs as sensory irritation or pulmonary irritation. Sensory irritation involves stimulation of trigeminal nerve endings located in the nasal mucosa. These sensory nerves are unmyelinated C fibers [2,3]. Upon stimulation by sensory irritants, these afferent nerves can release neuropeptides which convey a painful or burning sensation [4] and result in neurogenic inflammation and vasodilation in the nasal mucosa [5,6]. Pulmonary irritation stimulates vagal afferents either directly or through inflammation in the conducting airways and alveoli [7].

Alarie and colleagues [1,8] have developed a system to analyze breathing patterns in mice as a method to classify inhaled substances as sensory and/or pulmonary irritants. In mice, sensory irritants cause a characteristic pause which delays the onset of expiration, while pulmonary irritants cause a pause at the end of expiration. Both result in a measurable decrease in breathing frequency in the mouse model [7]. However, these breathing pattern changes are not identical in all species. For example, pulmonary irritants cause an increase rather than a decrease in breathing frequency accompanied with a decreased tidal volume in humans, guinea pigs and rats [1].

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The present manuscript summarizes data collected from our laboratories characterizing pulmonary responses of guinea pigs or rats to the inhalation of a variety of gases, vapors, or particulates associated with human respiratory disease in various occupational or environmental settings. Sensory irritation was determined as the release of neuropeptides from trigeminal nerve fibers extending to the nasal cavity. Pulmonary irritation was determined by measuring the infiltration of neutrophils into the bronchoalveolar airspaces as an indication of inflammation and by monitoring breathing rate elevation in guinea pigs or rats. In addition, pulmonary irritation was monitored by measuring neuropeptide release from sensory neurons innervating the conducting airways.

2. Methods

2.1. Experimental animals

Male guinea pigs [HsdPoc:DH] obtained from Harlan (Indianapolis, IN) and male Sprague–Dawley [Hla:(SD) CVF] rats obtained from Hilltop Lab Animals (Scottsdale, PA), monitored free of endogenous viral pathogens, parasites, mycoplasmas, Helicobacter and CAR Bacillus, were used for all experiments. The rats were acclimated for at least 5 days before use and were kept in filtered ventilated cages on Alpha-Dri virgin cellulose chips or hardwood Beta-chips as bedding, provided HEPA-filtered air, tap water, and feed [Teklad 7006 or Prolab 3500] ad lib. Facilities are AAALAC-accredited, specific pathogen-free, and environmentally controlled.

2.2. Neuropeptide responses

Neuropeptide responses to sensory or pulmonary irritants were assessed by measuring the tissue density of SP-containing nerve fibers located in the nasal or airway epithelium and SP levels and mRNA expression in specific nerve cell bodies in sensory ganglia (trigeminal ganglion neurons for the nasal cavity or nodose and jugular ganglia for the airways). Nerve fiber density was measured using digital imaging procedures to enhance and compute the pixel area of immunoreactive nerve fibers expressed as a percentage of the total epithelial area. Analysis of specific neurons projecting to the nasal and airway mucosa requires initial instillation of neural tracers into the nasal cavity or trachea with subsequent uptake by epithelial nerve terminals and finally transport and storage in the cell bodies of the sensory ganglia. Once the cells bodies contain the tracer, ganglia can be sectioned and immunostained or processed for in situ hybridization to reveal the neuropeptide or the associated mRNA. Peptide and message levels are measured using digital microscopy. These methods have been described in a previous publication [9].

2.3. Breathing rate

Breathing rate of guinea pigs or rats was determined using a method similar to that described previously [10]. Briefly, an animal was placed in a glass plethysmograph in which small differences in pressure were created by changes in the humidity, temperature, and compression of gas entering and leaving the lungs. These pressure changes, which were proportional to the animal's breathing pattern, were measured with a sensitive pressure transducer (Setra, Model 239). The spectral content of the pressure signal, which included the fundamental breathing frequency and harmonies, was computed with a high-resolution signal analyzer (B&K, Model 2033). Breathing rate for each animal was measured after 10% CO_2 flowed through the plethysmograph at a constant rate of 2 l/min for 4 min.

2.4. Pulmonary inflammation

Pulmonary inflammation in guinea pigs or rats was determined by measuring the number of polymorphonuclear leukocytes harvested by bronchoalveolar lavage (BAL). Animals were anesthetized with sodium pentobarbital, the trachea cannulated, and the lungs lavaged as described previously [11]. Briefly, lungs were lavaged with Ca^{2+}–Mg^{2+}-free phosphate-buffered saline (8 ml/lavage) until a total of 80 ml of fluid was collected. Lavage samples were centrifuged and the supernate discarded. BAL cells were then resuspended, centrifuged and resuspended again in HEPES–buffered solution (145 mM NaCl, 5 mM KC1, 1 mM $CaCl_2$, 5.5 mM glucose, and 10 mM HEPES: pH = 7.4).

Differential cell counts were made using an electronic cell counter equipped with a cell sizing attach-

ment (Coulter Counter and Channelizer) as described previously [11]. Polymorphonuclear leukocytes can be distinguished from other BAL cells by their characteristic cell volume [12].

3. Results

3.1. Neuropeptide responses to sensory irritants

In the nasal cavity, exposure by inhalation of 60 ppb toluene diisocyanate (TDI) for 2 h produces a time-dependent change in nerve fiber density in the nasal epithelium and in SP levels and expression of neurons projecting to the nasal cavity [13]. In the nasal epithelium, SP nerve fiber density is increased as early as 1 h after the end of the exposure, increasing to a peak at 24 h, and then returning to baseline by 96 h. At the same time, SP content and preprotachykinin mRNA both increase in trigeminal neurons projecting to the nasal mucosa (Fig. 1). Several studies have suggested that nerve growth factor (NGF) may play an important role in regulating SP expression in the

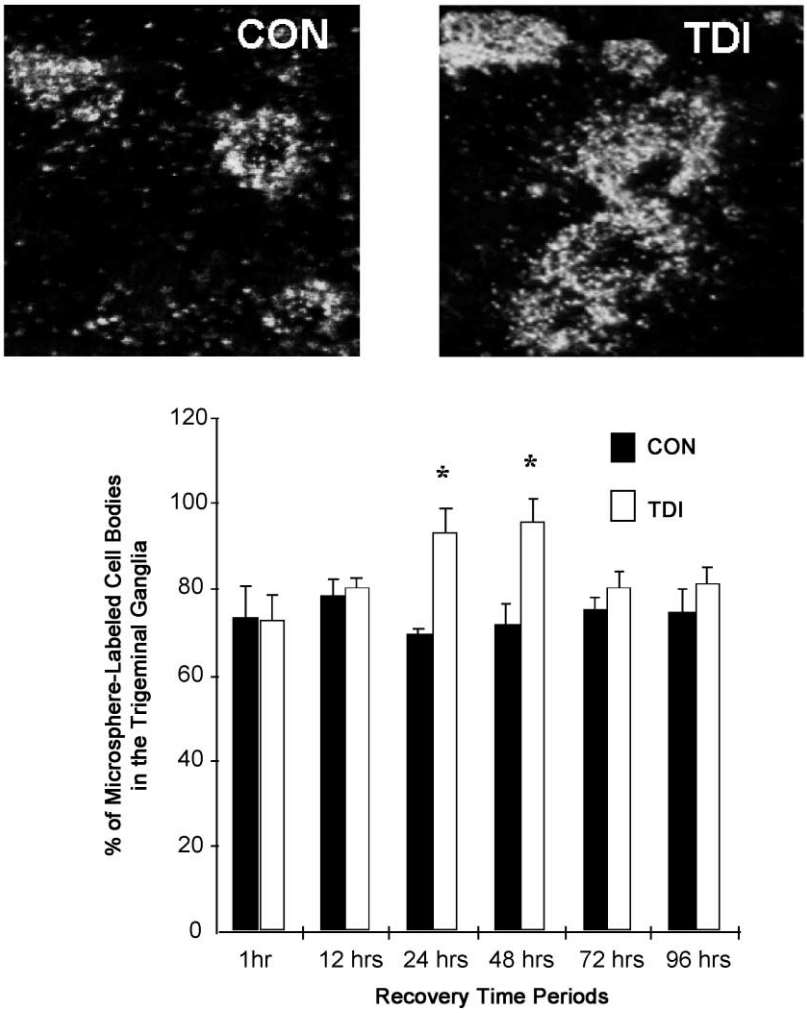

Fig. 1. TDI-induced changes of PPT mRNA grain density in trigeminal ganglion cells bodies innervating the nasal epithelium. Photomicrographs show in situ hybridization of PPT in trigeminal neurons that contained latex microspheres indicting their projection to the nasal epithelium (magnification 700 ×). The graph demonstrates the percentage of microsphere-labeled cell bodies that were positive for PPT mRNA. A significant increase in message levels in nasal neurons was present 24 and 48 h after TDI inhalation.

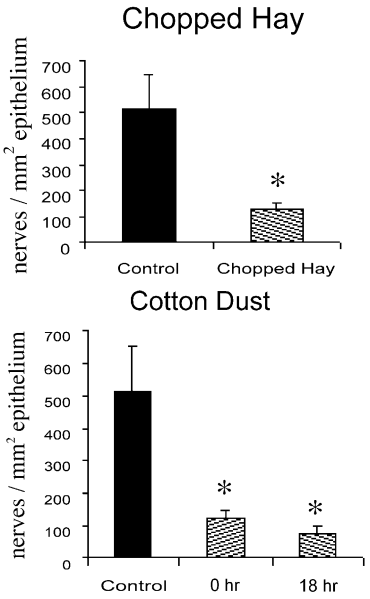

Fig. 2. Effects of chopped hay or cotton dust exposure on SP nerve fiber neuropeptides in the airway epithelium. Both chopped hay and cotton dust reduced SP nerve fiber density suggesting nerve activation and SP release.

respiratory system [14]. Preliminary evidence suggests that NGF levels in nasal lavage are enhanced after TDI instillation into the nasal cavity [15]. These data suggest that C fibers innervating the nasal cavity mediate responses to sensory irritants by generating action potentials traveling to the CNS, which evoke the expiratory pause probably through the release of glutamate, and by releasing SP locally in the nasal mucosa from sensory nerve terminals producing nasal inflammation. With respect to the influence of sensory irritants on breathing patterns, the neurotransmitter released in the CNS is of particular interest and is addressed in the discussion.

Nasal irritation is reported to occur in road workers and might be considered a sensory irritant. Rats exposed to asphalt fumes have increased neuropeptide levels in trigeminal neurons projecting to the nasal mucosa [16], supporting the possibility that asphalt fumes may act as a sensory irritant. There was no evidence that pulmonary irritation occurred (see below). The expiratory pause characteristics of sensory irritation have not been evaluated.

3.2. Neuropeptide responses to pulmonary irritants

Several organic dusts acting as pulmonary irritants also generate neuropeptide responses in the conducting airways. In these studies, guinea pigs were exposed to either cotton dust (11.6 mg/m^3 for 8 h) or chopped hay (5.9 mg/m^3 for 6 h) and sacrificed at 0 and 18 h, or 18 h post-exposure, respectively. Cotton dust and chopped hay both cause substantial reductions in SP nerve fiber density in airway epithelium (Fig. 2). Nerve density in airway smooth muscle for both neuropeptide Y (NPY) and the iNANC neuropeptide vasoactive intestinal peptide (VIP) are reduced after exposure to chopped hay (Fig. 3). Nerve fiber density of VIP in airway smooth muscle is reduced after cotton dust. These reductions in nerve fiber density suggest that neuropeptides had already been released by the time of analysis. All of the changes in smooth muscle occur only in the first 3–5 generations of intrapulmonary airways and were not observed in small distal airways or in the trachea or main stem bronchi, supporting an important role of the proximal airways in pulmonary inflammatory responses.

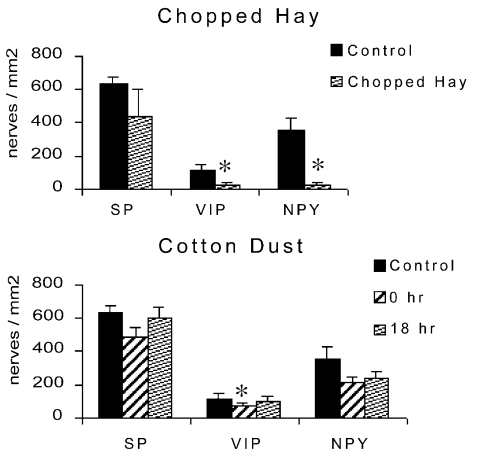

Fig. 3. Effects of chopped hay or cotton dust exposure on SP, VIP, and NPY nerve fiber density in airway smooth muscle of proximal intrapulmonary airways. Chopped hay caused reduced VIP and NPY fibers density and cotton dust caused a small but significant reduction in VIP immediately after exposure.

3.3. Pulmonary inflammation

Exposure to dust of organic origin has been associated with acute pulmonary reactions which include chest tightness, decreased pulmonary function and neutrophilic infiltration into the airspaces of the lung. Such a response has been termed "organic dust toxic syndrome" and has been described in cotton workers, dairy farmers, swine confinement workers, grain handlers, and wood workers [17]. Exposure of guinea pigs or rats to organic dusts is an excellent means to model this acute pulmonary inflammation as shown in Table 1. Inhalation of cotton dust, burnt hay, chopped hay, silage, or leaf/wood compost results in a dose-dependent increase in the number of PMN harvested by BAL [18–20]. In guinea pigs, this PMN infiltration peaks between 12 and 18 h post-exposure [18–20]. The inflammatory response to cotton dust in rats appears more rapid with PMN influx being maximal 6 h following exposure and decreasing thereafter [18]. In this regard, the guinea pig appears to be a better model of the time course of inflammation in workers exposed to organic dusts [20].

Organic dusts are often contaminated with bacteria and/or fungi [21,22]. Pulmonary reactions of workers have been associated with both the endotoxin and the β-glucan content of organic dusts [23–25]. Inhalation of endotoxin, the chemotactic peptide (*n*-formyl-methionyl-leucyl-phenylalanine, FMLP), or β-glucan results in pulmonary inflammation in the guinea pig model which is similar to that seen with exposure to organic dusts (Table 1). Indeed, evaluation of dusts from cotton grown in dry vs. wet regions of the country indicates a linear relationship between the inflammatory response of exposed guinea pigs and the endotoxin content of the dusts [29].

Pulmonary inflammation can also be demonstrated after inhalation of irritant vapors and gases (Table 1). Our laboratory has reported that a certain formulation of water repellent leather conditioner spray, which was associated with pulmonary disease in humans, causes significant bronchoalveolar inflammation in

Table 1
Pulmonary inflammation in response to inhalation of various gases, vapors and particles[a]

Agent	Exposure	Species	PMN	Ref.
Cotton dust	0	guinea pig	$0.46 \pm 0.04 \times 10^7$ cells/gp	[18]
	35 mg/m^3; 2 h	guinea pig	$4.00 \pm 1.23 \times 10^7$ cells/gp	[18]
	0	rat	$0.08 \pm 0.01 \times 10^6$ cells/rat	[18]
	35 mg/m^3, 2 h	rat	$4.47 \pm 1.00 \times 10^6$ cells/rat	[18]
Burnt hay	0	guinea pig	$0.10 \pm 0.01 \times 10^7$ cells/gp	[19]
	11 mg/m^3; 6 h	guinea pig	$1.50 \pm 0.50 \times 10^7$ cells/gp	[19]
Chopped hay	0	guinea pig	$0.33 \pm 0.03 \times 10^7$ cells/gp	[19]
	6 mg/m^3; 6 h	guinea pig	$2.66 \pm 0.60 \times 10^7$ cells/gp	[19]
Silage	0	guinea pig	$0.20 \pm 0.02 \times 10^7$ cells/gp	[19]
	8 mg/m^3; 6 h	guinea pig	$4.33 \pm 0.66 \times 10^7$ cells/gp	[19]
Leaf/Wood compost	0	guinea pig	$0.42 \pm 0.10 \times 10^7$ cells/gp	[20]
	30 mg/m^3; 4 h	guinea pig	$5.59 \pm 0.84 \times 10^7$ cells/gp	[20]
Endotoxin	0	guinea pig	$0.08 \pm 0.03 \times 10^7$ cells/gp	[26]
	4×10^4EU/m^3; 3 h	guinea pig	$3.31 \pm 0.69 \times 10^7$ cells/gp	[26]
FMLP	0	guinea pig	$0.15 \pm 0.01 \times 10^7$ cells/gp	[27]
	1 mg/m^3; 4 h	guinea pig	$1.38 \pm 0.35 \times 10^7$ cells/gp	[27]
β-Glucan	0	guinea pig	$0.30 \pm 0.02 \times 10^7$ cells/gp	[28]
	23 mg/m^3; 4 h	guinea pig	$3.72 \pm 0.57 \times 10^7$ cells/gp	[28]
Leather conditioner	0	guinea pig	$0.17 \pm 0.06 \times 10^7$ cells/gp	[30]
	2.5 mg/m^3; 2 h	guinea pig	$0.92 \pm 0.39 \times 10^7$ cells/gp	[30]
Asphalt fume	0	rat	$1.25 \pm 0.01 \times 10^6$ cells/rat	[32]
	20 mg/m^3; 6 h/day; 5 days	rat	$0.79 \pm 0.06 \times 10^6$ cells/rat	[32]
Ozone	0	rat	$0.40 \pm 0.04 \times 10^5$ cells/rat	[31]
	2 ppm; 3 h	rat	$3.30 \pm 1.10 \times 10^5$ cells/rat	[31]

[a] Animals were lavaged 18 h post-exposure except for the rats exposed to cotton dust which were sacrificed 6 h post-exposure. Pulmonary inflammation was monitored as the increase in polymorphonuclear leukocytes (PMN) obtained by bronchoalveolar lavage.

guinea pigs [30]. In addition, exposure of rats to ozone results in acute pulmonary inflammation characterized by an 8-fold increase in the yield of PMN after BAL [31]. In contrast, exposure of rats to relatively high concentrations (20 mg/m^3; 6 h/day for 5 days) of asphalt fume generated under road paving conditions ($\approx$150 °C) does not result in inflammatory recruitment of PMNs into the bronchoalveolar airspaces [32].

3.4. Breathing rate elevation

As predicted for pulmonary irritants, agents which cause pulmonary inflammation should result in an increased breathing frequency and a decrease in tidal volume in guinea pig and rat models [7]. Organic dusts, which are potent initiators of pulmonary inflammation, result in significant increases in breathing rates (Table 2). As with the inflammatory response, this enhancement of breathing frequency after cotton dust inhalation peaks between 12 and 18 h post-exposure in guinea pigs, while a more rapid response is seen in rats (a maximal effect observed immediately after exposure) [18]. The importance of pulmonary inflammation to this increase in breathing rate has been verified by Castranova et al. [33]. They report that depletion of peripheral neutrophils prior to inhalation of cotton dust not only inhibits PMN infiltration into the bronchoalveolar airspaces but decreases the enhancement of breathing rate by 75%. As with pulmonary inflammation, the breathing rate response of guinea pigs to inhalation of endotoxin, FMLP or β-glucan mimics that for exposure to organic dusts (Table 2). Indeed, a linear relationship exists between breathing rate elevation in guinea pigs and the endotoxin content of inhaled cotton dusts [29].

As with pulmonary inflammation, an increase in breathing frequency is observed after inhalation of

Table 2
Increased breathing rate in response to inhalation of various gases, vapors and particles[a]

Agent	Exposure	Species	Breathing rate	Ref.
Cotton dust	0	guinea pig	152 ± 4	[18]
	35 mg/m^3; 2 h	guinea pig	217 ± 19	[18]
	0	rat	170 ± 4	[18]
	35 mg/m^3; 2 h	rat	265 ± 12	[18]
Burnt hay	0	guinea pig	153 ± 5	[19]
	11 mg/m^3; 6 h	guinea pig	256 ± 15	[19]
Chopped hay	0	guinea pig	153 ± 5	[19]
	6 mg/m^3; 6 h	guinea pig	302 ± 16	[19]
Silage	0	guinea pig	144 ± 3	[19]
	8 mg/m^3; 6 h	guinea pig	203 ± 11	[19]
Leaf/Wood compost	0	guinea pig	134 ± 4	[20]
	30 mg/m^3; 4 h	guinea pig	186 ± 10	[20]
Endotoxin	0	guinea pig	120 ± 15	[26]
	4×10^4EU/m^3; 3 h	guinea pig	330 ± 38	[26]
FMLP	0	guinea pig	136 ± 2	[27]
	1 mg/m^3; 4 h	guinea pig	192 ± 5	[27]
β-Glucan	0	guinea pig	133 ± 4	[28]
	23 mg/m^3; 4 h	guinea pig	170 ± 23	[28]
Leather conditioner	0	guinea pig	133 ± 2	[30]
	2.5 mg/m^3; 2 h	guinea pig	277 ± 9	[30]
Asphalt fume	0	rat	149 ± 2	[32]
	20 mg/m^3; 6 h/day; 5 days	rat	150 ± 2	[32]
Ozone	0	rat	183 ± 6	[31]
	2 ppm; 3 h	rat	238 ± 8	[31]

[a] Breathing rate were measured in the presence of 10% CO_2 at the following times post-exposure: cotton dust (guinea pigs) — 12 h; cotton dust (rats) — 0 h; burnt hay, chopped hay and silage — 12 h; leaf/wood compost — 12 h; endotoxin — 18 h; FMLP — 0 h; β-glucan — 18 h; leather conditioner — 18 h; asphalt fume — 18 h; ozone — 0 h.

leather conditioner or ozone. In contrast, inhalation of asphalt fumes, which fails to cause an increase in lavagable PMN, does not elevate breathing rate in a rat model (Table 2).

4. Discussion

One of the common features of both sensory irritant responses, originating in the nose, and pulmonary irritant responses, originating in the airways, is the activation of sensory (afferent) nerve fibers. When activated by irritants, sensory fibers generate action potentials that travel to the brain stem and communicate by neurotransmitter release, usually glutamate, with second order neurons to eventually produce changes in breathing pattern [34]. Glutamate is an amino acid neurotransmitter long recognized as the most abundant excitatory neurotransmitter in the brain. Glutamate is present in neurons of the nodose and jugular ganglia [35] and is released from central projections that synapse with neurons in the nucleus of the tractus solitarius [36]. By binding to NMDA and non-NMDA receptors on NTS neurons, glutamate mediates reflexes that control breathing [37] and could conceivably mediate rapid, shallow breathing by reflex activation of C-fiber afferents [37]. The co-localization of glutamate and SP has not yet been established in morphologically identified airway neurons. However, recent studies suggest that SP released by activation of bronchopulmonary C-fiber afferents in the NTS alters breathing rate [34]. Since irritants also activate C fibers, this represents a possible mechanism regulating breathing responses to sensory and pulmonary irritants.

The other part of an irritant response, mediated by sensory C fibers and occurring through the local release of neurotransmitters (most notably substance P and neurokinin A), takes place within the mucosa of the nasal cavity or airway wall [38]. These tachykinins are typically associated with the process of neurogenic inflammation characterized by vasodilation and plasma extravasation [39–41]. SP is localized within cell bodies of the sensory ganglia and in the peripheral terminals of sensory nerves associated with arteries, veins, mucous glands, and epithelium [42,43]. The release of SP from these excitatory nonadrenergic, noncholinergic (eNANC) sensory nerve terminals has been linked to airway hyperresponsiveness [44], increased airway vascular permeability [45] and chemotaxis of inflammatory cells [46].

The possible involvement of SP and tachykinins in the pathogenesis of airways diseases is supported by several studies. Ollerenshaw and coworkers [47] demonstrated that SP nerve fiber density was increased in airway smooth muscle of severe asthmatics. A more recent study showed that the SP and CGRP nerve fiber density was increased in human airway epithelium from subjects with persistent non-productive cough [48]. SP synthesis and mRNA levels in sensory neurons of nodose ganglion are increased after antigen challenge in ovalbumin sensitized guinea pigs [49]. We have shown that neuronal levels of SP and PPTmRNA are increased in sensory trigeminal neurons innervating the nasal cavity of rats exposed to toluene diisocyanate [13]. These studies imply that increased levels of SP in airway nerves may contribute to altered airway function.

In addition to monitoring neuropeptide release from conducting airways, pulmonary irritation was monitored as the combination of pulmonary inflammation and elevated breathing rate in guinea pig or rat models. It has been proposed that inflammation can stimulate vagal afferents in the bronchoalveolar region of the lung, which results in a reflex increase in breathing frequency and a decrease in tidal volume [7]. Support for the direct involvement of PMN recruitment in the elevation of breathing rate is provided by experiments which demonstrate that the breathing rate response to cotton dust is dramatically reduced when PMN recruitment is depressed by depletion of peripheral leukocytes with cyclophosphamide pretreatment [33]. Results from our laboratory, measuring breathing rate and inflammation, indicate that the following occupational agents can be classified as pulmonary irritants: cotton dust, burnt hay, chopped hay, silage, leaf/wood compost, endotoxin, FMLP, β-glucan, leather conditioner, and ozone. Measurement of neuropeptide release from conducting airways for cotton dust and chopped hay confirm this conclusion. In contrast, asphalt fume does not appear to be a pulmonary irritant. Measurement of neuropeptide release from nasal sensory neurons indicate that asphalt fume is a sensory irritant. Toluene diisocyanate (TDI), methyl isocyanate and smoke from wood or polyvinlychloride plastics are other occupational exposures, which increase breathing rate in the

guinea pig model and would thus be classified as pulmonary irritants [50–53].

An extensive review has been presented by Alarie and colleagues [1] describing the use of the mouse model to evaluate the irritant potency of numerous agents found in industrial settings. Computerized analysis of breathing patterns of mice can be used to distinguish sensory irritation from pulmonary irritation [8]. Using this method, methyl isocyanate has been shown to induce both sensory and pulmonary irritation [51]. Our neuropeptide results with nasal sensory neurons support the conclusion that toluene diisocyanate is a sensory irritant [13], while breathing rate increases indicate that it is also a pulmonary irritant [50]. Indeed, in conducting airways, neurogenic inflammation and airway hyperreactivity to TDI is mediated in part through release of SP from C fibers [54,55]. Similarly, a machining fluid as well as many of the constituents of this synthetic fluid have been shown to be both sensory and pulmonary irritants [56].

In summary, many gases, vapors, or particles found in occupational and/or environmental settings can act as irritants. In guinea pigs and rats, sensory irritants are characterized by stimulation of neuropeptide release from sensory nerves in the nasal mucosa, while pulmonary irritants are characterized by recruitment of PMN into the bronchoalveolar airspaces and elevation of breathing frequency as well as neuropeptide release in conducting airways. In mice, analysis of breathing patterns has been employed to determine the sensory vs. pulmonary components of irritant responses to inhalation of various agents.

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