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ASPHALT EXPOSURE ENHANCES NEUROPEPTIDE LEVELS IN SENSORY NEURONS PROJECTING TO THE RAT NASAL EPITHELIUM

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Asphalt fumes have been reported to produce nasal irritation in road workers. Since inhaled irritants can increase substance P (SP) production in airway neurons, the effects of asphalt fumes on SP production in trigeminal ganglia (TG) sensory neurons innervating the nasal mucosa were investigated. The effects of asphalt fumes on nasal mucosal innervation were examined by measuring SP and calcitonin-gene-related peptide (CGRP) levels in rat TG neurons projecting to the nasal epithelium. Female Sprague-Dawley rats were exposed to asphalt fumes at 16.0 ± 8.1 mg/m^3 for 5 consecutive days, 3.5 h/d. Inflammatory cells were measured in nasal cavity lavage fluid. SP and CGRP immunoreactivity (IR) was measured in the cell bodies of trigeminal ganglion sensory neurons projecting to the nasal cavity. A significant increase in neutrophils and macrophages was observed after asphalt fume exposure indicating an inflammatory response in the nasal cavity. The percentage of SP-IR neurons increased significantly in the asphalt-exposed rats, and the proportion of CGRP-IR neurons was also elevated following asphalt exposure. These results indicate that exposure to asphalt fumes produces inflammation and increases the levels of SP and CGRP in TG neurons projecting to the nasal epithelium. The findings are consistent with asphalt-induced activation of sensory C-fibers in the nasal cavity. Enhanced sensory neuropeptide release from nerve terminals in the nasal cavity may produce neurogenic inflammation associated with nasal irritation following exposure to asphalt fumes.

Asphalt stone composite is the major paving product in the United States, accounting for over 90% of roadway paving. The road paving industry employed 300,000 workers as of 1990 (Asphalt Institute [AI], 1990). Derived

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from processing petroleum crude oil, asphalt is a complex mixture of high-molecular-weight organic compounds, including aromatic hydrocarbons and sulfur-nitrogen and oxygen-based heteromeric compounds (Hanley & Miller, 1996). The composition of asphalt varies, depending on the crude oil source and end usage. Application temperatures for paving asphalt range between 135 and 163 °C, producing bitumens, viscous solid or liquid by-products of petroleum refining to produce fumes. Depending on temperature, these fumes may contain polyaromatic hydrocarbons (PAHs), some of which are known human carcinogens (IARC, 1983, 1985). The immunotoxic potential of PAH has been documented in both animal and human studies (Schnizlein et al., 1987; Karakaya et al., 1999). Asphalt fumes may also be responsible for nonmalignant lung diseases such as bronchitis, emphysema, and asthma (Hansen, 1991; Maizlish et al., 1988) through activation of cytochrome P-450 isozyme CYP1A1 (Ma et al., 2002). The current threshold limit value (TLV) set by the American Conference of Governmental Industrial Hygienists (ACGIH) for asphalt fumes is $5\,mg/m^3$ for the 8-h time-weighted average (TWA) (ACGIH, 1991). National Institute of Occupational Safety and Health site studies involving asphalt paving workers list nasal, throat, and eye irritation as frequently reported symptoms (Hanley & Miller, 1996).

Airway responses to irritant inhalation are mediated in part by sensory and autonomic nerve fibers in the respiratory airways (Barnes, 1986). Irritants, including cigarette smoke, formalin, histamine, and sulfur dioxide, are known to activate sensory nerve fibers in the upper respiratory airway mucosa, inducing neurogenic inflammatory responses (Lundberg et al., 1987). The rat nasal mucosa receives sensory innervation from nerve cell bodies of the trigeminal ganglia (TG) (Grunditz et al., 1994). Sensory neurons projecting to the nasal mucosa are branched, small-diameter, capsaicin-sensitive C-fibers that can be activated by noxious chemical, mechanical, or thermal stimuli (Allen, 1924; Anton & Peppel, 1991). Upon activation with chemical stimuli such as capsaicin, the pungent agent in peppers, these fibers release neuropeptides, including substance P (SP) (Jancso et al., 1967). SP, an 11-amino-acid peptide, is localized within cell bodies of the TG and in the peripheral terminals of sensory nerves located near arteries, veins, mucous glands, and epithelium (Lundblad, 1984). Upon release, SP launches a receptor-mediated inflammatory response characterized by mucous secretion, plasma extravasation, and vasodilation (Jancso et al., 1967; Lundberg & Saria, 1983). Although SP has been implicated as the primary contributor in upper airway sensory responses (Baraniuk et al., 1991), the neuropeptide, CGRP, is costored and coreleased with SP (Lee et al., 1985; Lundberg et al., 1985). Nerve endings containing CGRP are extensively located along the walls of arterioles and veinules and upon release, CGRP produces long-lasting vasodilatory effects (Stjarne et al., 1989; Zhao & Tao, 1994).

Studies suggest that airway irritants affect neuropeptide levels in sensory neurons. Inhalation of either silica or toluene diisocyanate (TDI) has been demonstrated to transiently increase SP production in TG neurons projecting

to the nasal epithelium (Hunter et al., 1998, 2000). Nerve fibers in the airways and the neuropeptides they release may be involved in regulating the inflammatory responses to inhaled irritants.

The objective of the present study was to determine if inhalation of asphalt fumes activates sensory neurons innervating the nasal cavity. The proportion of TG neurons innervating the nasal epithelium that expresses SP and CGRP following asphalt fume inhalation was investigated. It was hypothesized that nasal irritation and inflammation following asphalt fume inhalation are due in part to enhanced neuropeptide production in TG neurons innervating the nasal cavity.

METHODS

Animals

Female Sprague-Dawley rats (Hla: [SD]CVF) weighing 200–250 g purchased from Hilltop Lab Animals (Scottsdale, PA) were verified by serology to be free of endogenous viral pathogens, parasites, mycoplasms, *Helicobacter*, and cilia-associated respiratory bacillus. Rats were acclimated in an AAALAC-accredited, specific-pathogen-free and environmentally controlled animal facility for 2 wk prior to initiation of inhalation exposures. When not in the inhalation chamber rats were kept in filtered, ventilated cages with Alpha-Dri cellulose chips (Shepard Specialties Papers, Kalamazoo, MI) and hardwood Beta-chips (Northeastern Products Corp., Warrensburg, NY) as bedding; provided with HEPA-filtered air, autoclaved Prolab 3500 diet (Purina Mills, St. Louis, MO) and tap water ad libitum; and housed under controlled light-cycle (12 h light/12 h dark) and temperature (22–24 °C) conditions.

Experimental Design

Rats were exposed to either asphalt fume or ambient air ($n = 7$ for both groups). In the experimental group, rats were exposed to 16.0 ± 8.1 mg/m^3 (mean ± SD) of asphalt fume for 5 consecutive days (3.5 h/d). The major sources of variability of fume concentration were when cans of asphalt were changed and day-to-day variation. A lethal dose of sodium pentobarbital (0.2 g/kg body weight, ip) was used to euthanize the rats 18 h after the last exposure. The right and left nasal cavities were lavaged simultaneously and cytospin slides were prepared for differential cell counts. The TG were then removed and processed for SP and CGRP immunocytochemistry.

Asphalt Exposure System

An asphalt fume generator (Heritage Research Group, Indianapolis, IN) was purchased and modified to produce an asphalt aerosol and gas suitable for small-animal exposure studies. Hot performance grade asphalt was purchased (PG 64-22, Asphalt Materials, Inc., Indianapolis, IN) and heated to 170 °C in a temperature-controlled kettle. The hot asphalt passed through

a needle valve, which regulated the flow. As the asphalt left the fume generator, its weight was measured with a digital scale. This value was acquired by the computer, and the asphalt mass flow rate was calculated by dividing the change in weight over the change in time. This flow rate signal was used in a feedback loop to adjust a stepper motor connected to the needle valve to achieve the desired flow rate of 150 g/min onto a 6-in-wide, 24-in-long, flat, stainless steel plate having a 1.3-degree slope with respect to horizontal. At the entrance to the plate, the asphalt was 160 °C. A 500-W heater underneath the plate maintained a temperature gradient of 35 °C along the length of the plate to simulate cooling under road paving conditions. The asphalt-covered plate was enclosed within a stainless steel chamber in which conditioned HEPA-filtered air passed through at a rate of 20 L/min. A mixture of air with the aerosol and gases released from the asphalt surface was removed from the chamber and conducted through a 1/2-in stainless steel pipe, heated to 150 °C with three 500-W heating tapes, into a 0.25-m^3 whole-body animal exposure chamber. Rats were exposed to asphalt fumes for 3.5 h/d during 5 consecutive days. Light-scattering measurements (Personal Data Ram, PDR-1000AN, MIE, Bedford, MA) of the asphalt aerosol within the animal exposure chamber were recorded in addition to gravimetric samples, which were made using a Teflon filter (PTFE, 0.45 µm pore size, SKC, Eighty Four, PA) and pump (Gilair5, Gilian, Sensidyne, Clearwater, FL) at a flow rate of 1.0 L/min. The light-scattering measurements were used in a computer feedback control system to modulate the diluent air that was mixed with the asphalt fume from the generator to keep the aerosol component of the asphalt fume constant (25 mg/m^3). The gas component of the fume was monitored with a photo ionization detector (MiniRae2000, RAE Systems, Sunnydale, CA), and gas samples were collected by pulling the filtered fume through an XAD-2 amberlite polyaromatic resin (number 226-30-06, SKC, Eighty Four, PA) with a constant-flow pump (Giliar5, Gilian, Sensidyne, Clearwater, FL) at a rate of 1.0 L/min. The XAD-2 samples were analyzed by mass spectroscopy to determine fume composition, and the results have been described elsewhere (Wang et al., 2001). Temperature and humidity within the exposure chamber were monitored (HMP 233, Vaisala, Woburn, MA) and recorded throughout each exposure period. The average temperature and relative humidity were 26 °C and 40%, respectively. Control animals were housed in similar chambers and exposed to HEPA-filtered conditioned air maintained at the same temperature and relative humidity as the environment within the asphalt exposure chambers.

Rhodamine-Labeled Latex Microsphere Instillation

Ten days prior to sacrifice, the rats were anesthetized with an intraperitoneal injection of sodium brevitol (25 mg/kg body weight). The anterior and posterior regions of the right and left nasal cavities were each instilled with 4 µl of rhodamine-labeled latex microspheres as previously described (Hunter & Dey, 1998). The microspheres were delivered using a 10-µl Hamilton syringe with plastic tubing covering the tip. The tubing was marked at 0.8 and 1.3-cm

lengths to allow correct positioning into the anterior and posterior nasal regions. Even distribution of the instilled material over the entire nasal mucosa was achieved by rotating the rats in a circular pattern around the anterior-posterior axis five times after microsphere instillation. The rhodamine-labeled latex microspheres were selectively taken up by sensory nerve endings in the nasal epithelium and then retrogradely transported to the corresponding cell bodies in the TG.

Nasal Lavage

The rats were overdosed with sodium pentobarbital (0.2 g/kg ip) and the lower jaw was removed. A syringe with plastic tubing covering the needle was inserted into the posterior nares and sealed by manual pressure. Both sides of the nasal cavity were simultaneously lavaged with sterile phosphate-buffered saline (PBS) until 20 ml was collected from the nostrils.

The lavage fluid was centrifuged at 1500 rpm (352 × g) for 10 min. The supernatant was discarded, and the pellet was resuspended in 1.0 ml PBS. Cells were plated on slides at a density of 1.5×10^5 cells/ml using a Cytospin (Shandon Scientific, Ltd., Cheshire, UK) at 400 rpm (18.06 × g) for 4 min. The slides were processed using the Hema 3 manual staining system (Biochemical Sciences, Inc., Swedesboro, NJ) for Wright-Giemsa stain. In total, 100 cells were classified as neutrophils, macrophages, eosinophils, basophils, lymphocytes, and epithelial cells using a light microscope (Olympus AX70) with a 40× magnification objective.

Tissue Removal and Preparation

The right TG were removed by cutting distal to the division of the ophthalmic, maxillary, and mandibular branches of the trigeminal and at the junction of the trigeminal nerve (V) emerging from the ganglion (Hunter & Dey, 1998). The tissue was immediately fixed in picric acid–formaldehyde fixative consisting of 2% paraformaldehye, 15% saturated picric acid, and 0.15 *M* phosphate buffer at 4 °C (Stefanini et al., 1967). After 3 h the tissue was rinsed twice with 0.1 *M* phosphate-buffered saline containing 0.3% (v/v) Triton X-100 (PBS-Tx, pH = 7.8). After the second rinse, the tissues remained in the PBS-Tx overnight at 4 °C. The TG were oriented on corks so the first section would be taken from the ventral surface. The tissue was covered with Tissue Tek OCT compound (Sakura, Torrance, CA), frozen in isopentane cooled by liquid nitrogen, and stored in airtight plastic bags at −80 °C.

Continuous-serial cryostat sections (12 μm thickness) of the entire TG were made as previously described (Hunter & Dey, 1998). Every fifth section was collected on one of three gelatin-coated coverslips. The first coverslip had sections 1, 6, 11, . . . , the second coverslip had sections 2, 7, 12, . . . , the third coverslip had sections 3, 8, 13, . . . , and so on until the entire ganglion was sectioned. The first two coverslips were used for SP immunocytochemistry and the third for CGRP immunocytochemistry.

Immunocytochemistry

Immunocytochemical procedures for localizing SP and CGRP immunoreactive neurons were identical to those previously described (Dey et al., 1990). Cryostat sections on gelatin-coated coverslips were covered with either rabbit anti-SP (diluted 1:200) or rabbit anti-CGRP (diluted 1:100) (Peninsula, Belmont, CA) primary antiserum. The coverslips were incubated in a humid chamber at 37 °C for 30 min, then rinsed 3 times with PBS-Tx+1% (w/v) bovine serum albumin (PBS-Tx-BSA), allowing 5 min per rinse. The sections were then covered with a diluted secondary antiserum, fluorescein isothiocyanate-labeled goat anti-rabbit immunoglobulin (Ig) G (ICN Pharmaceuticals Inc., Costa Mesa, CA) diluted 1:100 in PBS-Tx-BSA and the coverslips were incubated again at 37 °C for 30 min. The coverslips again were rinsed 3 times for 5-min increments in PBS-Tx-BSA. Fluoromount (Southern Biotechnology, Birmingham, AL) was used to mount the coverslips onto glass slides. The sections were observed using an Olympus AX70 fluorescence microscope equipped with fluorescein (excitation 495 nm and emission 520 nm) and rhodamine (excitation 540 nm and emission 580 nm)

Analysis of SP and CGRP Immunoreactivity

SP immunoreactivity (SP-IR) and CGRP immunoreactivity (CGRP-IR) in the TG cell bodies innervating the nasal epithelium were evaluated by direct observation. Without knowledge of the experimental grouping, neurons containing rhodamine-labeled latex microspheres were identified. Using the rhodamine filter, the presence of microspheres was used as a criterion that axons of the identified cell body projected to the nasal epithelium. The same cell body was then visualized with a fluorescein filter and classified as either SP-IR positive or negative. The percentage of SP-IR–positive neurons was determined by dividing the positive SP-IR neurons by the total number of microsphere-labeled neurons.

A second method of analysis was also used to evaluate just SP levels in TG neurons. The intensity of fluorescence of the cell bodies innervating the nasal cavity was determined, converted to a mean gray value (MGV) using digital image analysis, and compared against a threshold value to distinguish between positive and negative cell bodies. The system was calibrated using an InSpeck Green (505/515) microscope image intensity calibration kit (Molecular Probes, Eugene, OR). Once the neurons of interest were identified using the rhodamine filter, a black-and-white image was captured with a SPOT digital camera (Diagnostic Instruments, Inc., Sterling Heights, MI), and the perimeter of cell bodies containing microspheres was traced using Optimus, version 6.5, image analysis software (Media Cybernetics, L.P., Silver Spring, MD). Using the same field, an identical image was captured with the fluorescein filter and the outline of the cell bodies was superimposed. A mean gray value (MGV) was calculated for each neuron of interest using Optimus software. Neurons with an MGV <50 were considered negative and neurons with an MGV ≥ 50 classified as SP-IR.

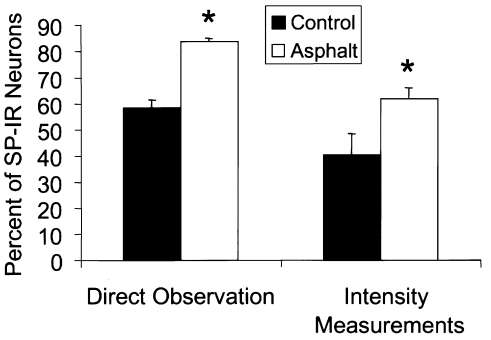

FIGURE 1. The percentage of SP-IR TG neurons innervating the nasal epithelium detected using both direct observation and intensity measurements by digital analysis following asphalt fume or ambient air (control) exposure. The results are from female Sprague-Dawley rats exposed to an asphalt fume concentration of aerosol particulate of 16.0 ± 8.1 mg/m^3, 3.5 h/d for 5 consecutive days. Asterisk denotes significant asphalt-induced change relative to controls ($p \leq .05$); $n = 7$ for each group.

The cutoff from negative to positive was based on an initial survey of several neurons in the TG directly observed to be either positive or negative and then digitally analyzed. The percentage of SP-IR neurons innervating the nasal epithelium was determined by dividing the positive SP-IR (MGV $\geq$ 50) microsphere-containing neurons by the total number of microsphere-labeled neurons.

Statistical Analysis

The means and standard errors were calculated for the percentage of SP-IR and CGRP-IR neurons in the TG and nasal lavage cell differentials. A Student's *t*-test was run with significance set at $p \leq .05$.

RESULTS

As determined by direct observation, the frequency of SP-IR–positive neurons following asphalt exposure ($83.7 \pm 1.4\%$) was significantly increased from the control group ($58.5 \pm 2.9\%$) (Figure 1). The asphalt group also showed a significant increase in the percentage of CGRP-IR–positive neurons over the control group, with values of 65.3 ± 2.0 and 49.7 ± 6.8, respectively (Figure 2). The increase in SP-IR following asphalt exposure was confirmed by a second method of evaluation involving threshold analysis of intensity measurements. The SP-IR of microsphere-containing neurons was determined by converting the immunofluorescent intensity to an MGV. Neurons with an MGV $\geq$ 50 were categorized as SP positive. Using digital image analysis, the percentage of SP-positive neurons in the control group ($40.5 \pm 8.1\%$) was found to be significantly lower than that for the asphalt group ($61.9 \pm 4.5\%$; Figures 1 and 3).

The nasal lavages showed the presence of inflammatory cells in response to inhalation of asphalt fumes (Figure 4). The percentage of neutrophils (5.3 ± 1.6) in the nasal lavage fluid of asphalt-exposed animals was increased

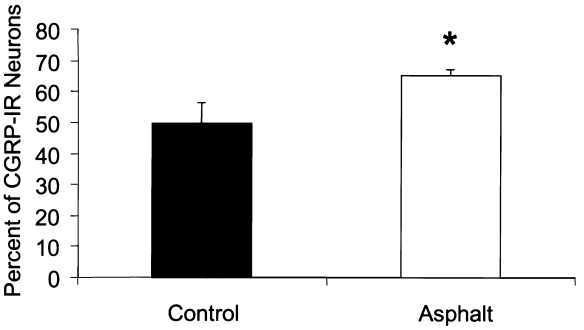

FIGURE 2. The percentage of CGRP-IR TG neurons supplying the nasal epithelium detected using direct observation after asphalt fume or ambient air (control) exposure. The results are from female Sprague-Dawley rats exposed to an asphalt fume concentration of 16.0 ± 8.1 mg/m^3, 3.5 h/d for 5 consecutive days. Asterisk denotes significant asphalt-induced change relative to controls ($p \leq .05$); $n = 7$ for each group.

4.1-fold over controls (1.3 ± 0.5). The asphalt-exposed group also had a significantly increased number of macrophages (22.0 ± 8.1%) in the nasal lavage fluid compared to control animals (2.4 ± 0.8%). The asphalt-exposed and control animals also had a large number of epithelial cells in the nasal lavage (61.7 ± 6.2% and 90.9 ± 3.1%, respectively, data not shown).

DISCUSSION

Past studies have investigated the role of asphalt in cancer, nonmalignant lung diseases, and renal disease (Ma et al., 2002; NIOSH, 2001). The present study is unique, being the first to evaluate the involvement of neuropeptides in the nasal cavity following asphalt inhalation. Retrograde transport of rhodamine-labeled latex microspheres instilled into the nasal cavity was used to identify cell bodies of sensory neurons in the TG that projected to the nasal cavity (Hunter & Dey, 1998). Immunocytochemical processing of TG cell bodies containing microspheres was the first step in the evaluation of SP-IR and CGRP-IR.

Our study compared two different methods of neuropeptide measurement, direct observation, and threshold analysis. Using both methods, it was determined that in the TG neurons innervating the nasal epithelium SP-IR were significantly increased following asphalt fume exposure over controls. Intensity measurements (40.6 ± 8.1% for control and 61.9 ± 4.5% for asphalt exposed) indicated fewer numbers of positive cells compared to direct measurements (58.5 ± 2.9% and 83.7 ± 1.3%, respectively). The threshold analysis method converts the fluorescent intensity of a TG neuron into a mean gray value, which eliminates the subjectivity that may occur using the naked eye. Although both methods have limitations, both are sufficient for detecting changes in neuropeptides. Direct observation is an established method for determining SP-IR in TG neurons following irritant inhalation (Hunter et al.,

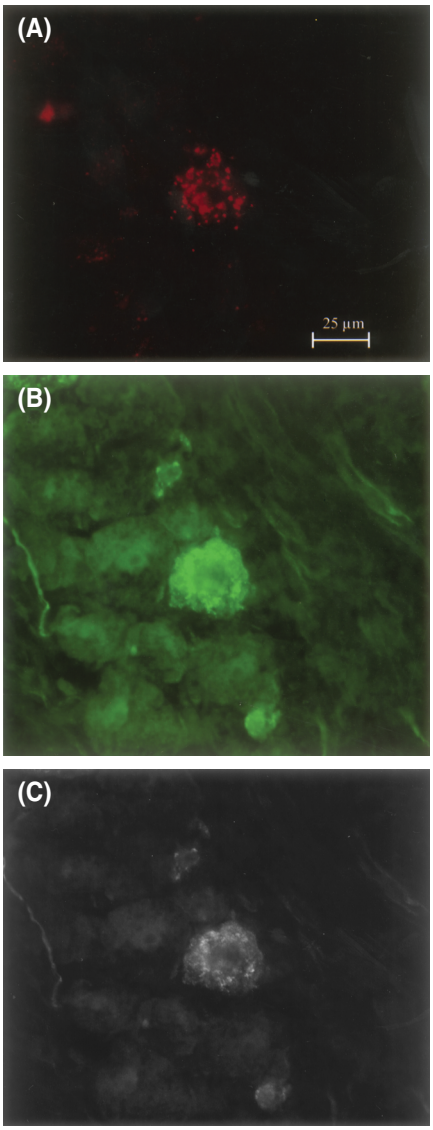

FIGURE 3. Images were obtained from a female Sprague-Dawley rat exposed to an asphalt fume concentration of 16.0 ± 8.1 mg/m^3, 3.5 h/d for 5 consecutive days. (A) The picture was taken with a rhodamine filter to identify neurons containing rhodamine-labeled latex microspheres, which are known to innervate the nasal epithelium. (B, C) Taken using a fluorescein filter except (B) was taken in color while (C) was taken in black and white. In (C), the microsphere-labeled neuron was traced and the MGV was calculated to be 73.1, indicating it was positive for SP-IR.

1998, 2000), but the use of digital images and image processing to determine positive and negative neurons reduces possible observer bias.

Previous studies demonstrated a link between neuropeptides, irritants, and inflammation. Inhalation of dust or chemical irritants produced rhinitis, nasal

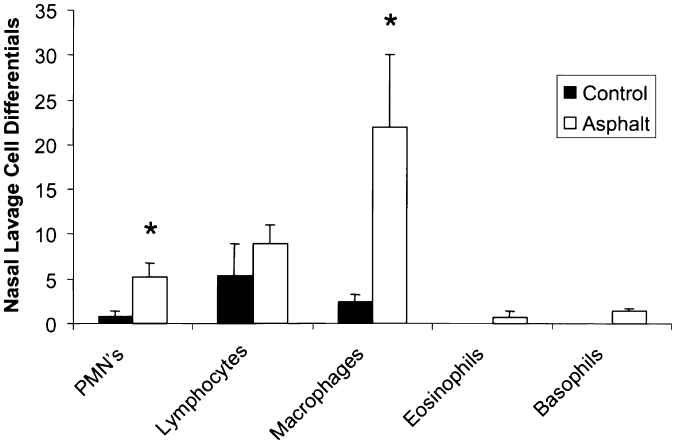

FIGURE 4. The nasal lavage cell differentials (% of total cells) from asphalt and ambient air (control) exposed rats. The results are from female Sprague-Dawley rats exposed to an asphalt fume concentration of 16.0 ± 8.1 mg/m^3, 3.5 h/d for 5 consecutive days. The mean for each cell type reported with an asterisk denotes significant asphalt-induced change relative to controls ($p \leq .05$); $n = 7$ for each group.

and upper airway irritation, and obstructive airway disease (Seixas et al., 1992; Vandenplas et al., 1993; Brugsch & Elkin, 1963). In rats exposed to inhaled silica for 6 mo, the proportion of SP-IR neurons innervating the nasal epithelium increased 8.7-fold compared to controls (Hunter et al., 1998). Increased SP-IR in microsphere-containing TG neurons was detectable in rats 48 h after toluene diisocyanate (TDI) exposure (Hunter et al., 2000). The latter studies also analyzed the level of preprotachykinin (PPT) messenger RNA (mRNA) in TG sensory neurons innervating the nasal epithelium and found an increase in message after both silica and TDI, exposure. Since PPT mRNA codes for SP, the increase in SP content is probably attributed to an increase in transcription of the PPT gene and translation of PPT mRNA.

The increase of SP-IR and CGRP-IR in TG neurons following asphalt exposure demonstrates enhanced synthesis and suggests an increase in subsequent release of SP and CGRP from nerve fibers in the nasal epithelium. Following synthesis in the TG cell bodies, neuropeptides undergo anterograde axonal transport to corresponding sensory-nerve terminals innervating inflamed tissue (Buck et al., 1999). In previous studies, SP levels in epithelial nerve terminals rapidly increased following TDI, suggesting enhanced anterograde-axonal transport of neuropeptides. After peaking 24 h following TDI exposure, the levels of SP in epithelial nerves steadily decreased and returned to control levels by 96 h, suggesting that SP was released into the surrounding nasal tissue (Hunter et al., 2000). The neural response to inhaled irritants has been termed neurogenic inflammation and is characterized by increased vascular permeability, plasma extravasation, edema, and inflammatory-cell chemotaxis, all of which are mediated primarily by increased SP release from sensory nerve endings

(Jancso et al., 1967). In the present study, the asphalt-induced elevation in SP-IR neurons was also associated with an increase in the number of intranasal neutrophils and macrophages. Epithelial damage and shedding and inflammatory cell influx are common sequelae following irritant inhalation (Harkema, 1990). Studies using capsaicin, the pungent agent in peppers, provided the first evidence that sensory nerves mediated neurogenic inflammation. Application of capsaicin to the nasal mucosa depletes SP from sensory nerve endings but has no effect on parasympathetic or sympathetic nerves (Lundblad et al., 1983). The release of SP from sensory nerve endings in the nasal mucosa is believed to be part of a protective reflex mechanism, which limits irritants from gaining access to the lower airways (Lundblad, 1984).

Nasal irritation has been reported among asphalt road crew workers (Hanley & Miller, 1996). When extrapolated over 8 h, the asphalt fume concentration ($10.8 \pm 1.2\ mg/m^3$, 3 h/d) used in an initial study was actually less than the American Conference of Governmental Industrial Hygienists (ACGIH) current threshold limit value (TLV); therefore, it is possible that neuropeptide levels may be elevated in asphalt workers with concomitant airway inflammation (Sikora et al., 2001). Inhaled asphalt fume particles may stimulate C-fibers in the nasal epithelium, resulting in neuropeptide production in the corresponding TG neurons and subsequent release from collateral sensory nerve endings in the nasal mucosa. Such a response in asphalt workers requires further investigation.

In conclusion, SP and CGRP levels are increased in upper airway sensory neurons of rats chronically exposed to asphalt fumes. These neuropeptides are involved in neurogenic inflammation, which may explain the influx of neutrophils and macrophages into the nasal cavity and their elevation in nasal lavage fluid. These findings suggest that asphalt fumes can be added to the growing list of chemicals known to stimulate C-fibers in the nasal cavity. In road construction workers, chronic asphalt exposure may activate sensory nerves in the nasal epithelium, leading to an increase in neuropeptides and resulting in nasal inflammation and irritation.

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