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Pulmonary neutrophil recruitment and bronchial reactivity in formaldehyde-exposed rats are modulated by mast cells and differentially by neuropeptides and nitric oxide

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Abstract

We have used a pharmacological approach to study the mechanisms underlying the rat lung injury and the airway reactivity changes induced by inhalation of formaldehyde (FA) (1% formalin solution, 90 min once a day, 4 days). The reactivity of isolated tracheae and intrapulmonary bronchi were assessed in dose–response curves to methacholine (MCh). Local and systemic inflammatory phenomena were evaluated in terms of leukocyte countings in bronchoalveolar lavage (BAL) fluid, blood, bone marrow lavage and spleen. Whereas the tracheal reactivity to MCh did not change, a significant bronchial hyporesponsiveness (BHR) was found after FA inhalation as compared with naive rats. Also, FA exposure significantly increased the total cell numbers in BAL, in peripheral blood and in the spleen, but did not modify the counts in bone marrow. Capsaicin hindered the increase of leukocyte number recovered in BAL fluid after FA exposure. Both compound 48/80 and indomethacin were able to prevent the lung neutrophil influx after FA, but indomethacin had no effect on that of mononuclear cells. Following FA inhalation, the treatment with sodium cromoglycate (SCG), but not with the nitric oxide (NO) synthase inhibitor L-NAME, significantly reduced the total cell number in BAL. Compound 48/80, L-NAME and SCG significantly prevented BHR to MCh after FA inhalation, whereas capsaicin was inactive in this regard. On the other hand, indomethacin exacerbated BHR. These data suggest that after FA inhalation, the resulting lung leukocyte influx and BHR may involve nitric oxide, airway sensory fibers and mast cell-derived mediators. The effect of NO seemed to be largely restricted to the bronchial tonus, whereas neuropeptides appeared to be linked to the inflammatory response, therefore indicating that the mechanisms responsible for the changes of airway responsiveness caused by FA may be separate from those underlying its inflammatory lung effects.

Keywords: Formaldehyde; Sensory fibers; Lung inflammation; Bronchial responsiveness

Introduction

The development of airway inflammatory disorders such as asthma, emphysema and bronchitis, associated with increased morbidity and mortality, has been correlated with exposure to a class of air pollutants including particulate matter and chemical substances such as formaldehyde (FA) (Green-Mckenzie and Hudes, 2005; Lambert et al., 2003). These agents in turn can damage the airway epithelial lining and shift the local immune balance, thereby facilitating the induction of persistent inflammation, and most conceivably the onset of chronic inflammatory diseases such as asthma (Handzel, 2000; Dales and Raizenne, 2004).

Asthma is characterized by pulmonary cellular infiltration and airway hyperresponsiveness, the latter being globally related to the toxic effects of mediators released in lungs by

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alveolar macrophages, neutrophils, eosinophils and mast cells (Bochner and Busse, 2005). Epidemiological evidence shows that the indoor and outdoor pollutant FA can trigger asthma (Delfino, 2002; Mendell and Heath, 2005). This compound is commonly found in car engine exhausts, foam insulation materials, floor coverings, office furniture, sterilizing agents and cigarette side stream (Harris et al., 1981; Krakowiak et al., 1998). It is also widely used in the cosmetic and textile industry (Carlson et al., 2004) as well as in medical and biological settings (Mizuki and Tsuda, 2001).

Due to its high chemical reactivity, the exposure to FA elicits pulmonary cell recruitment and increased microvascular leakage in the upper airways of animals (Lundberg and Saria, 1983; Cassee et al., 1996; Ito et al., 1996), which is closely related to what is observed in asthmatic patients (Bochner and Busse, 2005). In addition, FA can induce cancer in the nasal mucosa and upper airways (Conolly et al., 2004; Zhong and Que Hee, 2004), and modify the airways sensitivity to allergens (Wilhelmsson and Holmstrom, 1992; Riedel et al., 1996).

One of the major cells involved in the antigen-induced hyperresponsiveness is the mast cell (e.g. anaphylaxis and atopic asthma). Upon activation, these cells release a number of mediators that share a wide spectrum of inflammatory and regulatory functions in the airways and lungs (Galli et al., 2005).

The stimulation of sensory fibers exacerbates the inflammatory process by releasing neuropeptides such as the constrictor substance P and the potent vasodilator, calcitonin gene related peptide (CGRP) (Groneberg et al., 2004). CGRP, along with nitric oxide (NO), constitutes a part of the inhibitory branch of the non-adrenergic non-cholinergic system (NANC). NO is generated by three nitric oxide synthase (NOS) isoforms and regulates a wide spectrum of physiological conditions, including the modulation of airway smooth muscle tone (Fischer et al., 2002). Other studies suggested that C sensory fibers have a transient role in mediating neuroimmune effects on target cells in the respiratory tract (Fox, 1999). Thus, neuropeptides are likely to play an important role in a broad class of environmental health problems resulting from the exposure to chemicals.

Since airway dysfunctions have recently been correlated with exposure to pollutants (Delfino, 2002; Jang et al., 2003), we thought that a link might exist between sensory nerves, mast cells and the effects of FA exposure. This hypothesis was investigated herein by studying the rat airway susceptibility and leukocyte changes in response to FA exposure. Accordingly, we examined the modulatory role played by mast cells on these changes; the putative involvement of afferent, capsaicin-sensitive fibers and of NO was also studied.

Material and methods

Animals and experimental design. Male Wistar rats (180–200 g) from our Departmental facilities were used. They were housed in groups of five per cage in controlled temperature (21–23 °C) and 12 h light/dark cycle conventional conditions, with free access to food and water. Experiments were carried out in compliance with the guidelines of the local Animal Care Committee.

A standard glass chamber (20 L) coupled to an ultrasonic nebulizer device (Icel, Brazil) was used to generate a constant airstream from an aqueous solution of formalyn diluted to 1% FA by wt. The final concentration of methanol (present in the parent medium to prevent FA polymerization) in the nebulized solution was 0.32%. Animals were exposed to FA during 30, 60 or 90 min daily sessions for four consecutive days. A separate set of inhalation-free, intact animals was used as the naive group. Groups of naive rats were similarly exposed to a 0.32% aqueous solution of methanol. The experiments were carried out 24 h after the last FA inhalation. All the animals were killed by exsanguination of abdominal aorta under deep anesthesia (choral hydrate >400 mg/kg, ip).

Bronchoalveolar lavage (BAL) and pulmonary leukocyte quantification. Lung inflammation was evaluated by means of cell countings in the bronchoalveolar lavage (BAL) fluid of control and FA-treated animals. BAL fluid was taken from killed rats according to De Lima et al. (1992). Briefly, tracheas were cannulated with a polyethylene tubing (1 mm inner diameter) and lungs washed by flushing with phosphate buffered solution (PBS, pH 7.4, 10 ml). The recovered BAL fluid was centrifuged (170×g for 10 min at 20 °C), and the resulting cell pellet was then resuspended in PBS (1 ml). The total cell number was microscopically determined in Neubauer chambers while the differential cell counts (minimum 400 cells) were carried out via cytospin preparations and staining with May–Grünwald–Giemsa solution (Mordelet-Dambrine et al., 1984).

Measurement of leukocytes in the peripheral blood, bone marrow and spleen. Peripheral blood samples were taken from the tail vein of anesthetized rats (choral hydrate, 400 mg/kg, ip) and then were diluted (1:20) in Türk's fluid (3% acetic acid). The total cell counts were performed as described above, and differential counts were quantified on blood smears stained according to Mordelet-Dambrine et al. (1984).

In a parallel set of experiments, the femurs of killed rats were removed and the epiphyses were cut transversely (Fialho de Araujo et al., 2003). The bone marrow cells were flushed out with PBS (5 ml) and the recovered lavage fluid was centrifuged (170×g, 10 min at 20 °C). The cell pellets were resuspended in PBS (1 ml), stained using crystal violet (0.2%) and quantified microscopically as described above. In addition, the spleen was also removed from all groups of rats and placed on a glass Petri dish containing 10 ml of RPMI-1640 culture medium (Sigma) supplemented with EDTA (0.5%, final concentration). The spleen was trimmed and immediately homogenized. Following centrifugation (170×g, 10 min at 4 °C), 3 ml of Gey's solution was added to the homogenate in order to remove the red blood cells (Fang et al., 2000). The remaining cells were counted and the suspension was diluted in 10 ml of PBS and centrifuged (170×g, 10 min at 4 °C) according to Fang et al. (2000). The supernatant was discharged, the cell pellet was resuspended in 1 ml of PBS and the total cells were counted using a Neubauer chamber.

In vitro reactivity of isolated tracheal and bronchial tissue to methacholine. The chest wall of killed rats was opened and the cervical trachea (corresponding to the first 3–5 cartilaginous rings closest to the larynx) and the lungs were carefully removed. The intrapulmonary bronchus was isolated and dissected out of the surrounding tissues, as described by De Lima and Da Silva (1998). Both the tracheal and the intrapulmonary bronchial rings were mounted under resting tensions of 2 g and 1 g, respectively, on stainless-steel hooks in 30-ml capacity organ chambers bathed in Krebs–Henseleit (KH) solution, which was continuously gassed with 95% O_2 and 5% CO_2, at 37 °C. Isometric tension generated to methacholine (MCh) was measured using a force displacement transducer and recordings were displayed on a chart recorder (Myobath, World Precision Instruments, USA). Isolated tissues were allowed to equilibrate for 40 min. Tissue viabilities were assessed by replacing KH solution with depolarizing KCl buffer (60 mM) in the bath and comparing the force contraction generated with those obtained previously. Thereafter, cumulative dose–response curves to MCh were constructed according to Van Rossum (1963).

Lung morphological analysis. Lung fragments from both treated and control groups were fixed in 4% paraformaldehyde in 0.1 M Sorensen phosphate buffer, pH 7.4, at 4 °C for 24 h and embedded in paraplast (Sigma, USA). Sections (5-μm thick) were prepared for morphological (light microscopy) analysis upon