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Partial C-fiber ablation modulates diphenylmethane-4,4′-diisocyanate (MDI)-induced respiratory allergy in Brown Norway rats

Jürgen Pauluhn *, Hans-Werner Vohr

Institute of Toxicology, BAYER HealthCare, Building no. 514, 42096 Wuppertal, Germany

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Abstract

Brown Norway (BN) rats were topically sensitized to polymeric diphenylmethane-diisocyanate (MDI) and challenged with MDI-aerosol approximately every 2 weeks over a time period of 2 months. Half of the sensitized animals were pretreated with capsaicin for partial C-fiber defunctionalization. After the fourth challenge inflammatory and pro-inflammatory factors in bronchoalveolar lavage (BAL) fluid and cells and physiological delayed-onset breathing patterns were analyzed. The latter endpoint was examined in the capsaicin pretreated group before and after each challenge. Findings were compared against naïve but repeatedly MDI-challenged BN rats. BAL-neutrophils, -protein, and -LDH as well as lung weights were significantly increased in the MDI-sensitized and challenged rats relative to the naïve, challenged control rats. With regard to these endpoints, capsaicin pretreatment did not affect the responsiveness to MDI-aerosol. In contrast, pro-inflammatory cytokines, the Th2 cell cytokine IL-4, and the CC-chemokine MCP-1 were significantly increased in BAL-cells of capsaicin pretreated and MDI-sensitized rats, whilst in the normal MDI-sensitized rats markedly less pronounced changes (if any) occurred. In the former group, IL-4 and MCP-1 were also significantly increased in the lung draining lymph nodes. Time-related increased frequencies of delayed-onset responses were observed in MDI-sensitized rats after subsequent MDI-challenges, however, differences between capsaicin pretreated and normal rats were not found. Despite the remarkable differences between normal and capsaicin pre-treated rats in the concentrations of pro-inflammatory and Th1-/Th2-cell specific cytokines, the inflammatory endpoints in BAL as well as the physiological measurements did not identify appreciable differences amongst these groups. This study included an ancillary study addressing the analysis of the modulating effect of capsaicin pre-treatment of naïve Wistar rats exposed for single 6 h to MDI-aerosol. The results indicated more pronounced changes on endpoints in the BAL-fluid of capsaicin-pretreated rats as compared to rats with intact C-fibers. This complex picture appears to suggest that C-fibers may modulate the allergic inflammatory response elicited by MDI-challenge. It appears that tachykinergic sensory C-fibers modulate the protective pathways against irritant-related lung inflammation and, similarly, also pro-inflammatory immunological factors modulating allergic inflammation. Although difficult to disentangle unequivocally the mechanisms involved, neuro-immunological factors may be important in triggering and maintaining this complex disease and cytokine/chemokine patterns may not necessarily predict the functional outcome of test.

Keywords: Diphenylmethane diisocyanate; MDI; Respiratory hypersensitivity; Lung function; Delayed responses; Penh; Neutrophilic inflammation; Asthma; Capsaicin; Cytokine fingerprinting

* Corresponding author. Tel.: +49 202 368909; fax: +49 202 364589.
E-mail address: juergen.pauluhn@bayerhealthcare.com (J. Pauluhn).

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1. Introduction

Diisocyanates are widely used in industry and are known to be involved as causative agents for occupational asthma (Chan-Yeung and Malo, 1994; Johnson et al., 2004). The pathogenesis associated with low-molecular-weight diisocyanates is not well understood, including the role of the dermal route of induction to initiate disease. The latency period in the reactions observed in specific challenge tests indicate immunological mechanisms, however, specific immunoglobulin E (IgE) antibodies have been identified only in 14–20% of the cases (Baur, 1983; Piirilä et al., 2000), suggesting that non-immunological factors may contribute to disease. Thus, the role of serum IgE in diisocyanate-induced allergic airway disease is still controversial. In this context, neural mechanisms and their interaction with inflammatory cells have been reported to be important modifying factors in the pathophysiology of asthma, commonly referred to as a neurogenic inflammation (Barnes, 1992; Bousquet et al., 2000; Joos et al., 1994). It has been suggested that C sensory fibers have a transient role in mediating neuroimmune effects on target cells in the respiratory tract (Fox, 1999; Scheerens et al., 1996; Kranefeld and Nijkamp, 2001; Hunter et al., 2000; Graham et al., 2001). Neurogenic inflammation is mediated by the release of neuropeptides subsumed as tachykinins, e.g. substance P, from capsaicin sensitive C-fibers in the rodent airway mucosa. Inflammation is characterized by increased vascular permeability, plasma extravasation, glandular secretion, and neutrophil chemotaxis. Stimulation of sensory nerves innervating the vascular bed of the tracheobronchial mucosa causes increased release of tachykinins resulting in vasodilation as well as to an enhanced production and release (pro)inflammatory mediators (Hunter et al., 2000; Kranefeld and Nijkamp, 2001). Many investigations of sensory nerve function have relied on the use of capsaicin, which acts through the vanilloid receptor. At the dose of capsaicin used in this study, defunctionalization of sensory nerves, including a partial depletion of several neuropeptides, that result in long-term loss of sensory nerve function have been described (Holzer, 1991; Chung et al., 1990; Lundblad, 1984; Lundblad et al., 1985; Morris et al., 1999; Stjarne et al., 1989). With regard to the ablation of C-fibers and responses to irritants appreciable differences across different rat strains were not observed (Takebayashi et al., 1998). Sterner-Kock et al. (1996) demonstrated that the capsaicin pretreatment of neonatal Wistar rats (examinations after 2 month) using a similar dosing regime as used in this study reduced the substance P content in lung tissue homogenates by 77% compared to normal rats. However, not all C-fibers belong to the capsaicin-sensitive class (Hunter et al., 2000) so that localized tachykinergic innervation from C-fibers subclasses may still be operative, despite capsaicin pretreatment.

In previous studies in the Brown Norway (BN) rat with the benchmark low-molecular weight asthmagenic chemical agent TMA (trimellitic anhydride) remarkable pathophysiological changes in inflammatory and physiological (breathing patterns) endpoints have been observed during an inhalation challenge, independent of whether the induction was topical or by inhalation (Pauluhn et al., 2002; Pauluhn, 2003; Zhang et al., 2004). To the contrary, similar methodological approaches failed to identify MDI as an asthmagenic compound when using a single inhalation-challenge regime. However, when using a repeated inhalation challenge regime, MDI was unequivocally identified as an asthmagenic substance both with regard to transiently occurring respiratory responses delayed in onset as well as by a number of inflammatory endpoints in bronchoalveolar lavage (BAL), however, only following topical rather than inhalation induction (Pauluhn, 2005; Pauluhn et al., 2005). So far, only a limited number of studies have evaluated the sequence of inflammatory events taking place after repeated, chronic inhalation challenges (Holgate et al., 2000; Palmans et al., 2000, 2002; Tomkinson et al., 2001; Leigh et al., 2002; Kips et al., 2003). At face value, the data obtained with MDI in the repeated challenge BN rat model appear to support a hypothesis favoring an immunological rather than a non-immunological pathophysiology. However, multiple challenge exposures to MDI-aerosol are required to phenotypically manifest asthma-like effects as shown in previous studies (Pauluhn, 2005; Pauluhn et al., 2005).

The objective of this study was to study the modifying impact of sensory nerves (C-fibers) that upon stimulation release neuropeptides through the axon reflex. Pro-inflammatory effects of these peptides also promote the recruitment, adherence, and activation of granulocytes that may further exacerbate neurogenic inflammation, i.e., plasma extravasation and vasodilation (Solway and Leff, 1991). The modifying role of capsaicin-induced sensory nerve defunctionalization on protein extravasation and the recruitment of inflammatory cells in BAL fluid was evaluated in a sensitization study using normal and capsaicin pretreated BN rats that were topical induced and repeatedly challenged with MDI-aerosol. This analysis included an evaluation of allergic respiratory responses delayed in onset. Moreover, pro-inflammatory cytokines/chemokines and inflammatory endpoints from bronchoalveolar lavage fluid and cells