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Bilateral vagotomy or atropine pre-treatment reduces experimental diesel-soot induced lung inflammation

D.S. McQueen [a,*], K. Donaldson [b], S.M. Bond [a], J.D. McNeilly [b], S. Newman [a], N.J. Barton [a], R. Duffin [b]

[a] University of Edinburgh, School of Biomedical Sciences, 1 George Square, Edinburgh EH8 9JZ, Scotland, UK
[b] MRC/University of Edinburgh, Centre for Inflammation Research, ELEGI Colt Laboratory, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK

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Abstract

To investigate the role of the vagus nerve in acute inflammatory and cardiorespiratory responses to diesel particulate (DP) in the rat airway, we measured changes in respiration, blood pressure and neutrophils in lungs of urethane anesthetized Wistar rats 6-h post-instillation of DP (500 μg) and studied the effect of mid-cervical vagotomy or atropine (1 mg kg^{-1}) pre-treatment. In conscious rats, we investigated DP, with and without atropine pre-treatment. DP increased neutrophil level in BAL (bronchoalveolar lavage) fluid from intact anesthetized rats to $2.5\pm0.7\times10^6$ cells ($n=8$), compared with saline instillation ($0.3\pm0.1\times10^6$, $n=7$; $P<0.05$). Vagotomy reduced DP neutrophilia to $0.8\pm0.2\times10^6$ cells ($n=8$; $P<0.05$ *vs.* intact); atropine reduced DP-induced neutrophilia to $0.3\pm0.2\times10^6$ ($n=4$; $P<0.05$). In conscious rats, DP neutrophilia of $8.5\pm1.8\times10^6$, $n=4$, was reduced by pre-treatment with atropine to $2.2\pm1.2\times10^6$ cells, $n=3$. Hyperventilation occurred 6 h after DP in anesthetized rats with intact vagi, but not in bilaterally vagotomized or atropine pre-treated animals and was abolished by vagotomy ($P<0.05$, paired test). There were no significant differences in the other variables (mean blood pressure, heart rate and heart rate variability) measured before and 360 min after DP. In conclusion, DP activates a pro-inflammatory vago-vagal reflex which is reduced by atropine. Muscarinic ACh receptors in the rat lung are involved in DP-induced neutrophilia, and hence muscarinic antagonists may reduce airway and/or cardiovascular inflammation evoked by inhaled atmospheric DP in susceptible individuals.

Keywords: Diesel particulate; Pulmonary; Vagal reflex; Neutrophilia; Inflammation; Muscarinic cholinoceptor; Atropine; Sensory nerves

Introduction

There is a well-documented association between air pollution and cardiorespiratory disease, and particulate material (PM) has been identified as the most potent component of the air pollutants (Donaldson et al., 2001, 2002; Brunekreef and Holgate, 2002; MacNee and Donaldson, 2003). Diesel exhaust particles (DP) are nanoparticles (diameter <100 nm) in the size range that are collected by the PM_{10} and $PM_{2.5}$ (μm) conventions (Kittelson, 1998). DP share properties with a number of other similar particle types, including the ability to generate inflammation via oxidative stress mechanisms that have been recently reviewed (Donaldson et al., 2005). Various epidemiological studies have investigated the relationship between PM and the increased rates of morbidity and mortality from cardiovascular and pulmonary diseases (e.g. Pope, 2000), and the cardiovascular effects of PM have been reviewed recently (Brook et al., 2004). Salvi and Holgate (1999) demonstrated that exposure of healthy humans to diesel exhaust evoked inflammatory responses, including enhanced transcription of cytokines and chemokines in lung biopsies. PM has been

Abbreviations: ACh, acetylcholine; AM, alveolar macrophage; BAL, bronchoalveolar lavage; DBP, diastolic blood pressure; COPD, chronic obstructive pulmonary disease; DP, diesel exhaust particulate matter; DMS, dimethylsulfoxide; HPA, hypothalamic–pituitary–adrenal; HR, heart rate; LPS, lipopolysaccharide; mAChR, muscarinic acetylcholine receptor; MBP, mean blood pressure; nAChR, nicotinic acetylcholine receptor; PBS, phosphate buffered solution; RARs, rapidly adapting receptors; PM, particulate matter; RMV, respiratory minute volume; SBP, systolic blood pressure; TNF, tumor necrosis factor.

* Corresponding author.
E-mail address: D.S.McQueen@ed.ac.uk (D.S. McQueen).

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reported to act directly on the autonomic nervous system, leading to changes in breathing, heart rate (HR) and HR variability (Stone and Godleski, 1999). Studies in mice also show that DP induces airway hyper-responsiveness (Ohta et al., 1999), and animal models are being used to investigate how particles affect cardiopulmonary function.

The respiratory tract from the larynx down to the smallest airways and alveoli is innervated by the vagus nerve, which conducts sensory and motor neural activity to and from the CNS, respectively, via distinct afferent and efferent nerve fibers (Widdicombe, 1985; Spina, 2002). Cytokines have been shown to stimulate afferent vagal fibers, triggering the release of immunomodulatory glucocorticoids (Sternberg, 1997), and it has been proposed that efferent vagal fibers may be an additional anti-inflammatory pathway: Borovikova et al. (2000) described a parasympathetic anti-inflammatory pathway after finding that acetylcholine (ACh) inhibited the release of tumor necrosis factor (TNF) evoked by lipopolysaccharide (LPS) in cultured macrophages. In the presence of the muscarinic ACh receptor (mAChR) antagonist atropine, ACh still inhibited TNF release, yet α-conotoxin reversed the inhibitory effects of ACh. This indicates that macrophage α-bungarotoxin-sensitive nicotinic receptors (nAChR) are primarily responsible for mediating the inhibitory effect of ACh on the LPS-induced TNF response in human macrophages. Bilateral cervical vagotomy in rats significantly increased the peak serum levels of the cytokine TNF in response to a lethal dose of LPS, while electrical stimulation of the efferent vagus nerve significantly decreased the amounts of TNF in the serum (Borovikova et al., 2000). This nicotinic 'cholinergic anti-inflammatory pathway' apparently reflexly monitors and adjusts the inflammatory response (Tracey, 2002).

If afferent and efferent fibers of the vagus nerve innervating the lungs do modulate the inflammatory response to DP via vago-vagal reflexes, then ACh released from parasympathetic nerve terminals will also affect mAChRs, in addition to any action the neurotransmitter has on nAChRs in the lungs. It is also possible that ACh generated from non-neuronal sources, such as cells of the immune system (Wessler and Kirkpatrick, 2001), could also act on AChRs in the airways, including mAChRs located on immune cells such as macrophages (de la Torre et al., 2005) and lymphocytes (Sato et al., 1999). We therefore tested the hypothesis that DP provoke acute pulmonary inflammation via actions involving muscarinic AChRs. A combination of bilateral vagotomy, which eliminates vago-vagal reflexes, and the muscarinic AChR antagonist atropine was used to study pulmonary inflammation and cardiorespiratory changes evoked by intratracheal instillation of DP in rats. A preliminary report on the respiratory effects of DP instillation has been published (McQueen et al., 2004).

Materials and methods

Laboratory animals and anesthesia. The experimental protocol was in accord with European Community guidelines approved by the local ethics committee and licensed under UK Home Office regulations. Adult male Wistar rats (Charles River, Margate, Kent; 228–500 g) were housed four per cage and fed *ad lib* on a standard diet.

Experiments in conscious animals. Rats were transiently anesthetized with a mixture of ketamine (50 mg kg^{-1}) and medetomidine (100 μg kg^{-1}) intraperitoneally and DP or vehicle instilled into the airway, with the aid of a laryngoscope to expose the trachea. Immediately following instillation, the anesthesia was reversed by atipamezole (190 μg kg^{-1} s.c.). Conscious animals were pre-treated with atropine (1 mg kg^{-1} i.p.) 30 min before DP or vehicle alone was administered, and the dose repeated 2 and 4 h post-instillation. Animals were killed 6 h after instillation by a single intraperitoneal (i.p.) injection of pentobarbital and the lungs lavaged, as described below.

Experiments in anesthetized animals (vagotomy and/or atropine). Animals were anesthetized with urethane (ethyl carbamate 25% w/v aqueous solution, 6 ml kg^{-1} i.p. single dose), and rectal temperature was maintained at 36–37 °C by a heating blanket connected to a thermistor probe in the rectum.

Ventilation and blood pressure (BP). A tracheal cannula was connected to a pneumotachograph and airflow, tidal volume and respiratory frequency were measured using an electrospirometer (MacLab). Arterial BP was measured from a catheter inserted into a common carotid artery (MacLab). Arterial blood samples (0.1 ml) were taken from a cannulated femoral artery for measurement of blood gas tensions and pH using a blood gas analyzer (Rapidlab 248, Bayer). The arterial oxygen tension was maintained at ~100 mm Hg by enriching the air the animal breathed with oxygen, which was necessary in a minority of experiments. A femoral vein was cannulated for the i.v. administration of drugs.

Experimental procedures. DP was dispersed in sterile saline at a concentration of 1 mg ml^{-1} and sonicated for 5 min (Soniprep 150, Sanyo). Sterile saline (0.9% w/v aqueous sodium chloride) or diesel, 0.5 ml, was instilled over a period of 15 s via a catheter with its tip positioned at the carina. In 16 animals the vagus nerve trunks were identified at mid-cervical level, gently separated from the carotid artery and sympathetic nerve trunk, and sectioned on both sides 30 min prior to instillation. In a further 27 animals the intact vagus nerves were sectioned 5 min before the end of the experiment, 6–7 h after instillation of DP or vehicle. Physiological variables were monitored throughout the six-hour period following instillation.

Atropine was injected 10–20 min pre-instillation (1 mg kg^{-1} i.v., repeated at intervals of 2 h) to block mAChRs without affecting nAChRs. The muscarinic agonist bethanechol was injected (100 μg i.v.) in some experiments to check the effectiveness of the atropine block. Bethanechol caused transient hypotension, which was completely abolished by the dose of atropine used.

Measurement of inflammatory cells in rat lungs following BAL (bronchoalveolar lavage). Six hours after DP or saline instillation anesthetized rats were killed, the lungs removed for bronchoalveolar lavage (BAL), and 8 ml sterile saline was syringed into the airway via the tracheal cannula. The lavage fluid was recovered by gentle aspiration, and three further lavages were performed. The fluid from these collections was combined and stored on ice before sedimentation of the cells. BAL cells were pelleted at 1200 rpm at 4 °C for 5 min. The supernatant from the first lavage was retained and stored as 500 μl aliquots at −80 °C until required for biochemical analysis. The supernatant fraction from the subsequent lavages was discarded. All cells were resuspended in 1 ml phosphate buffered solution (PBS). Cell counts were performed using a hemocytometer chamber (Sigma-Aldrich, Dorset, UK), and cell viability was assessed by trypan blue dye exclusion. Cytospin preparations were made using 10,000 cells centrifuged at 300 rpm for 3 min onto glass slides (Menzel-Glaser). The cells were fixed with methanol and stained using Diff Quick dyes. Differential leukocyte counts of the cytospins were performed with a minimum of 300 cells counted per slide.

Data analysis. Systolic (SBP) and diastolic (DBP) blood pressures were measured from the chart record using MacLab software, as was respiratory frequency and tidal volume. Cardiac interval was determined by measuring the R–R interval from ECG recordings obtained using a MacLab Bioamp and HRV software (Chart) over 30 s, or approximately 220 heart beats, before (time 0) and 360 min post-instillation. The coefficient of variation (%) ([standard deviation/mean]*100) was used to estimate the variability in inter-beat interval before (time 0) and at 5 and 360 min after tracheal instillation of saline or DP. Values for mean blood pressure (MBP) were computed using MacLab: MBP=