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Differences in the pattern of bronchoconstriction induced by intravenous and inhaled methacholine in rabbit

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ABSTRACT

We measured bronchoconstriction in central bronchi, and in small peripheral airways causing the emergence of ventilation defects (*VD*), through two delivery routes: intravenous (IV) and inhaled MCh, in 2 groups of rabbits (A: $n=5$; B: $n=4$), using synchrotron imaging of regional lung structure and ventilation. We assessed the effect an initial IV challenge on a subsequent inhaled challenge in group B. Inhaled MCh decreased central airway cross-sections (*CA*) by 13–22%, but increased *VD* area by 25–49%. IV MCh decreased *CA* by 44% but increased the area of ventilation defects (*VD*) by 13% only. An initial IV MCh challenge reduced regional ventilation heterogeneity following a subsequent inhaled MCh challenge, suggesting the role of agonist–receptor interaction in the response pattern. Heterogeneous agonist distribution due to uneven aerosol deposition could explain the different patterns of response between IV and inhaled routes. This mechanism could participate in the emergence of ventilation heterogeneities during bronchial challenge, or exposure to allergen in asthmatic patients.

1. Introduction

Inhaled methacholine (MCh) is commonly used to assess bronchial hyperresponsiveness (BHR) for the diagnosis of asthma (Juniper et al., 1981; Crapo et al., 2000). This is usually accomplished by measuring the overall airway response by spirometry, following MCh aerosol inhalation. The most common variable used to assess airway responsiveness in the clinical setting is forced expiratory volume in one second, which mainly reflects changes in central bronchi, but is rather insensitive to small airway constriction and the spatial heterogeneity of bronchoconstriction. However, bronchial smooth muscle constriction shows substantial regional heterogeneity following airway challenge in asthmatic patients, resulting in a heterogeneous regional ventilation distribution. The spatial heterogeneity of regional ventilation in response to airway challenge, has been demonstrated in human asthmatics by positron emission tomography (PET) (Harris et al., 2006) and magnetic resonance imaging (MRI) using hyperpolarized ^{3}He (Costella et al., 2012; Samee et al., 2003). Few preclinical studies have assessed the changes in regional ventilation distribution caused by MCh inhalation, using functional imaging techniques. Bronchoconstriction induced by MCh inhalation has been shown to produce clustered ventilation defects and a significant ventilation heterogeneity in sheep by PET imaging (Vidal Melo et al., 2005), in rabbit, using synchrotron imaging (Bayat et al., 2009a), and in mouse, using MRI with hyperpolarized ^{3}He (Thomas et al., 2012). Data both from these experimental studies and previous human studies suggest that clustered areas of poor regional ventilation, or "ventilation defects" (*VD*) arise from collective constriction or closure of peripheral airways. The mechanisms of the emergence of such ventilation defects remain poorly understood, despite their crucial importance not only for lung mechanics and gas exchange, but also for their potential impact on the distribution of inhaled medications.

Theoretical studies based on integrative computational models of the entire human bronchial tree, suggest that ventilation defects occur beyond a critical level of airway smooth muscle constriction, even with a homogenous activation of the airway smooth muscle (Venegas et al., 2005; Winkler and Venegas, 2012). These studies have suggested that the uneven distribution of the constricting agonist may not be an essential mechanism in the emergence of peripheral ventilation defects. In a previous study in rabbit, we observed that the route of constricting agonist delivery changes the pattern of airway response distribution, despite similar overall respiratory mechanical changes (Bayat et al., 2009a). In that study,

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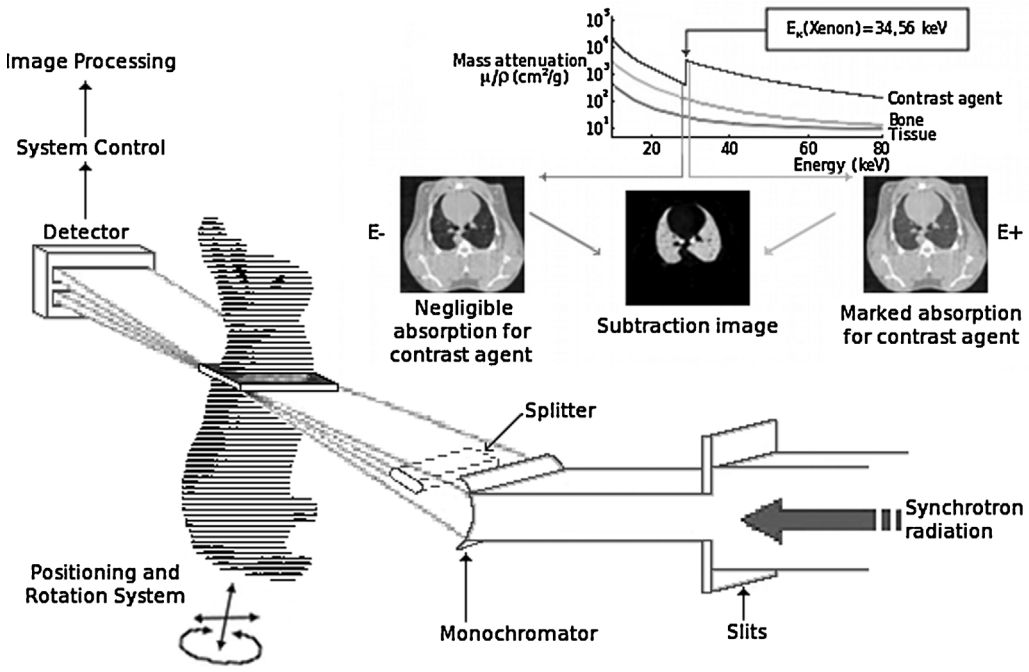

Fig. 1. Principles and experimental setup of K-edge subtraction imaging. At the Xe K-shell absorption edge (E_K), the attenuation coefficient of Xe increases by a factor of 5.4, while the changes in the attenuation coefficients of other elements remain negligible. The absorption CT images below and above the K-absorption edge show the anatomical structures. The logarithmic difference of these images yields the Xe density on an absolute scale, while features due to other structures are removed. The quantitative density due to either tissue or to Xe can therefore be calculated separately in each voxel, which provides both structural and functional data in the same set of images.

the inhaled route of bronchial challenge produced a significantly more heterogeneous distribution of regional ventilation with dramatically larger ventilation defects. We have recently found that aerosol particles of identical aerodynamic diameter than in our previous study, show significant deposition heterogeneity in rabbit (Bayat et al., 2011). These observations suggested that uneven deposition of the inhaled particles carrying a constricting agonist could substantially modify the spatial distribution of bronchoconstriction. This hypothesis is significant in the clinical setting, since uneven agonist distribution could contribute to the emergence of ventilation defects during bronchial challenge, or exposure to irritants or allergen, in patients.

In the present study, we tested the hypothesis that the distribution of the constricting agonist plays a role in the emergence of ventilation defects. To this end, we compared the magnitude of bronchoconstriction in central bronchi of different sizes, and in small peripheral airways causing the emergence of ventilation defects, through two routes of MCh challenge: intravenous (IV) infusion and inhaled.

To assess whether the observed patterns of response to MCh are specifically determined by agonist–receptor interactions, we compared the patterns of central and peripheral airway response within the same subject, through subsequent challenges through the infused and inhaled routes. A reduced patchiness of regional ventilation, following an initial IV infusion challenge, or tolerance to MCh, would be in favor of this hypothesis.

2. Methods

Animal care and procedures of the experiment were in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council and approved by the local institutional authorities. The experiments were performed on nine male New Zealand rabbits (average weight: 2.5 ± 0.1 kg; Elevage Scientifique des Dombes, Chatillon sur Chalaronne, France).

2.1. Animal preparation

A catheter was inserted in the marginal ear vein (22-gauge, Cathlon IV, Ethicon, Rome, Italy) after local anesthesia using 5% topical lidocaine (Emla, Astra-Zeneca, Rueil, France). Anesthesia was induced by IV injection of thiopental sodium (25 mg/kg IV, Nesdonal, Rhone-Poulenc-Rohrer, Paris, France). The animal was tracheostomized, and an endotracheal tube (no. 3.5, Portex, Berck sur Mer, France) was inserted and secured with a gas-tight seal. A catheter (22-gauge) was inserted into the left carotid artery for blood pressure monitoring and arterial blood sampling for blood gas analysis (Radiometer model 77, Acid Base Laboratory, Copenhagen, Denmark). The lower extremities were wrapped with bandage, and the animal was immobilized in a custom-made plastic holder in the vertical position. The chest wall and diaphragmatic motion were free of constraint. The forelimbs were fixed to the holder to keep them out of the image field and the lower limbs were securely maintained in the holder using foam.

Anesthesia was maintained by inhaled isoflurane (0.5–1.5%, Forene, Abbott, Paris, France). Paralysis was induced by IV vecuronium bromide (1.0 mg/h, Norcuron, Organon, Puteaux, France). A custom-made mechanical ventilator was used (Monfraix et al., 2005). The animals were ventilated in pressure-control mode, with a tidal volume (VT) of 7 ml/kg, and a respiratory rate adjusted in order to maintain a $PaCO_2$ close to 40 mmHg, as monitored by arterial blood gas analysis. Mean end-expiratory pressure was 1.5 cmH_2O. Gas flows were measured continuously and recorded using mass flow meters (Aalborg, Orangeburg, NY). During imaging, the inhaled gas mixture was automatically switched to xenon (Xe, 70%) and oxygen (O_2, 30%) using electromagnetic valves controlled by the image acquisition software.

Airway pressure (P_{rs}) was monitored continuously. Ventilatory gas flows were measured using a heated pneumotachometer (Hans Rudolph, Kansas City, MO). All monitored signals were amplified, digitized at 1000 Hz (PowerLab, ADInstruments, Oxfordshire, UK), and recorded on a computer. At the end of the experiment, the