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Bronchodilation induced by muscular contraction in spontaneously breathing rabbits: Neural or mechanical?

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ABSTRACT

The respective contribution of mechanical and neural mechanisms to the bronchodilation occurring during exercise is not fully identified in spontaneously breathing animals. The airway response to electrically induced muscular contractions (MC) was studied after vagal cold block in 9 spontaneously breathing rabbits. The forced oscillation respiratory system resistance (Rrs) was measured at vagal nerve temperatures 37 °C, 8 °C and 4 °C. Rrs was found to decrease significantly during MC in all conditions. The occasional occurrence of a deep breath was responsible for a sudden decrease in Rrs. However, when the deep breath was absent – after vagal cooling and in some experiments at 37 °C – the bronchodilation was frequently dissociated from the change in breathing pattern, most likely illustrating a neural mechanism. Altogether, while some bronchodilation may be ascribed to the mechanical stretching of the airways, Rrs decreasing with little change in breathing pattern is likely related to a reflex effect, possibly a sympathetic-borne mechanism.

1. Introduction

Airway function is dynamically determined in health and disease (Chapman et al., 2011), and the airway calibre may be impacted by a voluntary deep breath, a spontaneous sigh (Brusasco and Pellegrino, 2003), or increased ventilation from deeper or faster breathing (Gunst et al., 2001). A typical illustration is the bronchodilation that occurs during exercise in health and asthma (Crimi et al., 2002; Freedman, 1992; Milanese et al., 2009). The mechanical stretching of those airways subjected to parenchymal tethering during the hyperventilation is thought to be the primary mechanism. Indeed in bronchoconstricted subjects, the bronchodilation is similar during exercise and during voluntary isocapnic hyperventilation when the exercise pattern of increase in tidal volume is copied during the voluntary manoeuvre (Freedman et al., 1988).

On the other hand, there is evidence for a neural link to explain the airways response to increasing lung volume. In dogs, the extrathoracic trachea dilates in response to lung inflation, therefore independent of any lung – airway mechanical interaction (Widdicombe and Nadel, 1963). In humans, heart–lung transplant recipients challenged with methacholine do not bronchodilate in response to a deep breath, in contrast with control subjects (Glanville et al., 1988). When exercise is mimicked by electrically induced muscular contraction, a reflex increase in airway calibre has been shown in dogs with bilateral phrenic denervation, undergoing constant artificial ventilatory support (Kaufman et al., 1985). During the more physiological condition of spontaneous breathing, the mechanisms of airway adaptation to exercise are not easily ascertained because of the hyperventilation. However, a continuous estimation of airway calibre may be obtained by the respiratory resistance at “zero” flow using the forced oscillation technique at a single excitation frequency. Significant bronchodilation was thus demonstrated during electrically induced muscular contraction in spontaneously breathing rabbits. It was however not clear whether the vagal control was of physiological significance (Marchal et al., 2008). Should this be the case, then the bronchodilation during the mock exercise would be attenuated or blocked, when interrupting the airway cholinergic innervation, particularly in rabbits that show consistent parasympathetic bronchomotor tone (Karczewski and Widdicombe, 1969). The condition should be met at or below 8 °C where most traffic in myelinated fibres is interrupted (Paintal, 1967).

The aim of the study was therefore to characterize the airway response to induced muscular contractions in rabbits during cooling of the cervical vagi. The hypothesis was that significant vagally mediated bronchodilation during exercise would be inhibited by the neural block.

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2. Materials and methods

2.1. Animals

Nine New Zealand adult rabbits (weight range: 2.5–3.9 kg) were studied. Animal housing and experiments were performed according to the recommendations 86-609 CEE issued by the Council of the European Communities and under licenses from the “Ministère de l’Agriculture et de la Pêche” and the “Ministère de l’Enseignement Supérieur et de la Recherche” (A54518-03409) and supervision by the “Services Vétérinaires Départementaux de Meurthe et Moselle”.

2.2. General

The general procedure was similar to that described previously (Marchal et al., 2008). Briefly, the spontaneously breathing animal was placed on a heating pad, anaesthetised with a mixture of urethane (500 mg/kg), alpha-chloralose (50 mg/kg) and sodium borate (50 mg/kg) injected through an ear vein. Supplemental doses were given every 2 h. The trachea was incised, intubated and connected to a Fleisch no 0 pneumotachograph to measure airflow. Transrespiratory pressure was generated by a loudspeaker as a sine wave forcing signal at 20 Hz, and measured at a side port of the cannula. The respiratory resistance (Rrs) was computed oscillation per oscillation, as the real part of the complex ratio of transrespiratory pressure to flow. The hind limbs were equipped with stimulating electrodes taped over the skin covering the gastrocnemius lateralis and connected to an electrical stimulator. The sequence consisted in a train of eight 7.6 ms square waves (5–10 V) for 0.1 s repeated every 0.68 s. The stimulation was maintained for 30 s.

2.3. Vagal cold block

The cervical vagi were carefully dissected free from surrounding tissues and placed in a metallic gutter where the local temperature was controlled through Peltier elements (Thermoelectric cooler MI1025T, Marlow Industries Inc, Dallas, TX, USA) and could be decreased from 37 °C (θ_{V37}) down to 8 °C (θ_{V8}) in the main experiments. The temperature end point was chosen because it has been shown to suppress any significant bronchoconstriction induced by electrical stimulation of the vagi rostral to the cold block, and the cooling manoeuvre could be repeated without evident damage to the nerve (Schweitzer et al., 2011).

2.4. Protocol

A typical data acquisition included a baseline followed by electrically induced muscular contractions for 30 s (MC). At least 1 acquisition was performed at θ_{V37} and repeated at θ_{V8} in control conditions. These measurements were repeated following an aerosol of methacholine, titrated so as to approximately double the control Rrs (Marchal et al., 2008). A minimum of 10 min recovery was allowed to elapse between any 2 successive epochs. In some animals, MC’s were repeated after lowering the vagal temperature down to 4 °C.

2.5. Data analysis

The respiratory signals were displayed with a resolution of 0.05 s. Tidal flow was digitally integrated to volume using the trapezoidal method, after correcting for an electrical zero offset. To minimize Rrs short term fluctuations and to clarify Rrs change related to MC in the figures, Rrs is presented after moving average filtering with a period of 40 data points, i.e., 2 s.

The quantitative analysis was made using tidal volume (VT), breathing rate (BR), minute ventilation (VE) and Rrs computed breath by breath. The flow dependence in Rrs was eliminated by selecting, in each breath, the value closest to zero breathing flow at the end of expiration. In this way the airway calibre could be tracked continuously, independent of ventilation. The respiratory variables were averaged over the relevant study period (baseline or MC) and compared using a two way analysis of variance (STATVIEW 2 software abacus concepts, Berkeley, CA, USA). A statistical significance was retained for a p value <0.05. Data are expressed as mean + SEM unless otherwise indicated.

3. Results

Twenty nine MCs were obtained at θ_{V37} (13 controls and 16 methacholine) and 22 at θ_{V8} (9 controls and 13 methacholine). Eleven additional experiments were performed at 4 °C or less. In control experiments from θ_{V37} to θ_{V8}, there is an overall statistically significant increase in VT ($p < 0.01$), decrease in BR and Rrs ($p < 0.01$, Table 1).

The typical ventilatory response to MC is characterized by faster breathing, no significant change in VT (Table 1), an occasional deep breath at onset of the stimulation. Heightening the bronchomotor tone with methacholine apparently sensitizes the bronchodilatory effect of the latter (Fig. 1). Interestingly, in some experiments, Rrs decreases progressively throughout MC. On average, there is a significant decrease in Rrs, both at control and after methacholine ($p < 0.01$, Table 1).

At θ_{V8}, no deep breath is observed. One pattern of ventilatory response is slowing of breathing and apneusis at onset of MC, while Rrs decreases to a lower value throughout the stimulation period (Fig. 2). Another pattern of response is a transient early decrease in VT associated with a decrease in Rrs (Fig. 3). Overall during MC, VT does not change at control and decreases after methacholine ($p < 0.05$, Table 1). Also, VE is frequently observed to decrease transiently at onset of MC and to peak beyond the stimulation period, being therefore delayed rather than suppressed, and little significant hyperventilation is apparent during MC (Table 1). In contrast there is a statistically significant decrease in Rrs during MC, both at control ($p < 0.01$) and after methacholine ($p < 0.01$, Table 1).

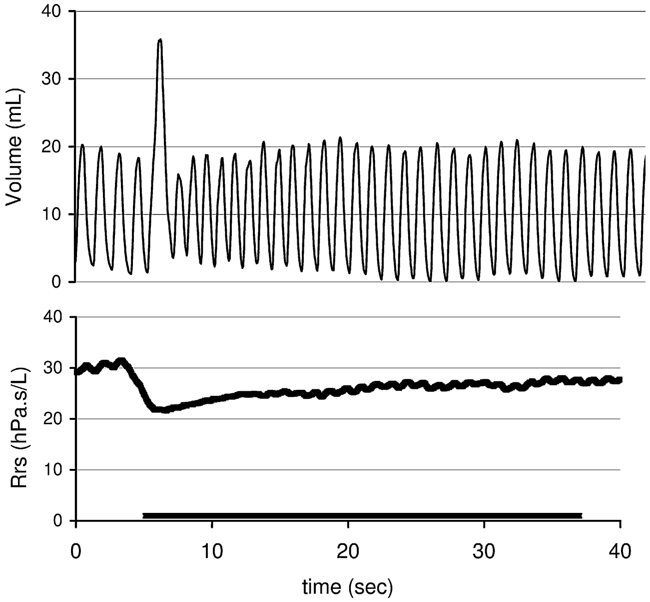

Fig. 1. Changes in lung volume and respiratory resistance (Rrs) in response to muscular contractions (MC, horizontal bar) after methacholine inhalation. The abrupt and large drop in Rrs corresponds to the initial deep breath which is followed by increased ventilation.