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Airway response to induced muscular contraction in spontaneously breathing rabbits

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Abstract

The airways are thought to dilate during exercise in humans but the time course and mechanisms of the response are not fully described. The aim of the study was to document changes in airway calibre during electrically induced muscular contractions (MC) in spontaneously breathing rabbits. Experiments were also performed after induced bronchoconstriction to assess the effect of change in breathing pattern on airway calibre during MC. Respiratory resistance (R_{RS}) was measured in 12 rabbits using the forced oscillation technique at 20 Hz before, during and after 30 s MC in control conditions and after methacholine induced bronchoconstriction (Mch). MC was associated with significant decrease in R_{RS} both at control and Mch. The MC induced increase in V_E occurred with significant albeit small change in mean tidal volume (V_T) at Mch but not control. An augmented breath (AB) occurred in 29/35 MCs and was usually associated with an abrupt drop in R_{RS}. The decrease in R_{RS} induced by AB was significantly larger at Mch compared with control. Passively inflating the lung after MC induced significantly larger decrease in R_{RS} than AB during MC. The data indicate bronchodilation by MC in spontaneously breathing rabbits. The mechanisms appear to include AB dependent airway wall stretching as well as removal of cholinergic input to the airway smooth muscle.

Keywords: Exercise; Bronchodilation; Bronchomotor tone; Methacholine; Airway calibre; Breathing pattern; Augmented breath; Airway wall hysteresis

1. Introduction

The airway response associated with exercise has been studied in humans and animals and bronchodilation, which is thought to represent the main change, is likely to relate to different causes (Beck, 1999; Crimi et al., 2002; Freedman, 1992; Kaufman, 1996). Neural mechanisms are based on the joint evidence of decreased lung resistance in response to both muscular contraction induced by motor nerve stimulation and electrical stimulation of types III–IV muscular fibres (Kaufman et al., 1985, Rybicki and Kaufman, 1985). The data have been obtained in paralyzed animals, therefore independent of any change in breathing pattern. Exercise however is usually associated with an increase in depth of breathing which may promote bronchodilation as a result of the mechanical interaction between lung parenchyma and conducting airway wall during stretching (Freedman, 1992). The mechanism is independent of exercise as a stimulus, since the volitional increase in tidal volume (V_T) has been shown to relax pharmacologically constricted human airways as well (Freedman et al., 1988; Stirling et al., 1983). In addition, a deep inhalation is able to reverse bronchomotor tone, although most evidence is based on studies in passive or voluntary lung inflation (see Brusasco and Pellegrino, 2003) rather than spontaneous or reflex induced augmented breaths (AB).

Development of integrated animal models would thus be of particular help to track and detail the time course of bronchomotor changes at exercise during spontaneous breathing and therefore to provide better insight into the mechanisms of this airway response (White et al., 2001). Measurements should not interfere with breathing or bronchomotor tone, should be reasonably insensitive to change in respiratory flow (de Bisschop et al., 2003) and should be able to document the time course of change in airway dimensions. The respiratory resistance by the forced oscillation technique (R_{RS}) has been used as a proxy to airway calibre, allowing the tracking of its time related change while minimizing flow dependent properties (Schweitzer et al.,

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2006). Significant ventilatory response to electrical stimulation of muscular nerves in rabbits (Tallarida et al., 1983) or muscles in sheep (Haouzi et al., 1997) has been documented. Such model provides the potential to characterize airway changes during spontaneous breathing in exercising animals, but to the best of our knowledge, little data are available. The rabbit was chosen because application of small pressure oscillations has been shown not to alter respiratory mechanics and measurements may easily be implemented with standard equipment (Peslin et al., 1994). In addition this species shares with humans the paucity of adrenergic nerve fibres to the airway smooth muscle (Canning, 2003).

The aim of the study was therefore to validate a rabbit model to assess bronchomotor change occurring when exercise conditions are reproduced by inducing muscular contraction. To more specifically test the hypothesis that increased stretch to conducting airways contributes to exercise induced bronchodilation, measurements were also performed following methacholine (Mch) inhalation. If the hypothesis is correct, then bronchodilation of exercise should be magnified during acutely induced contraction of airway smooth muscle which increases hysteresis of conducting airway wall.

2. Materials and methods

Twenty New Zealand adult rabbits (weight 3–5 kg) were studied.

2.1. Anaesthesia and surgical preparation

Anaesthesia was induced 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 skin of thighs and legs was carefully shaved and depilated. The rabbit was tracheostomized and intubated with a tracheal steel cannula. The animal was placed prone in a hammock suspended above the table, the semi-flexed hind limbs were in contact with the table top. Each hind limb was maintained with sets of springs connected to freely hanging lead weights. Rectal temperature was continuously monitored with an electrical thermistor (Physitemp Instruments, YSI 402 Clifton, NJ, USA) and maintained at 38 °C using a circulating warm water pad placed under the rabbit's belly.

2.2. Muscular contractions

Electrical stimulation was performed through surface electrodes (Electrodes autocollantes réutilisables, Saint Cloud International, Chantonnay, France) taped on the hind limb skin and connected to a S88 Grass stimulator (West Warwick RI, USA). Electrode placements were tested on different muscles during pilot experiments and stimulation of the gastrocnemius lateralis appeared to induce the most significant hind limb motion. 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. In each animal, a stimulus threshold was determined by observing the muscle area during a stepwise increase in intensity and was defined as the lowest intensity triggering visible contraction. The stimulus intensity was increased to an average of three times the threshold. In what follows MC refers to muscular contractions such as obtained in the current study.

2.3. Mch

Mch aerosol was delivered as previously described (Mazurek et al., 1995), with adaptation of the technique for spontaneously breathing rabbits. The aim was to at least double R_{RS}. The challenge started at an initial concentration of 1 mg/mL and additional doubling doses given as necessary.

2.4. R_{RS} measurement

The technique to assess breath × breath change in airway calibre has been described previously (Schweitzer et al., 2006) and was adapted for measuring rabbits. Briefly, a horn driver type loudspeaker excited the respiratory system at a single frequency of 20 Hz. A three-way connector allowed the rabbit to breathe through a high inertance circuit which was continuously flushed to prevent CO_2 accumulation. Airway flow was measured at tracheal cannula using a Fleisch # 0 pneumotachograph (Metabo, Hepalinges, Switzerland) and airway pressure at a side port of the cannula. R_{RS} was computed oscillation cycle per oscillation cycle from the real part of the complex airway pressure—flow ratio. In order to avoid flow dependence of R_{RS}, the final calculation included only those R_{RS} values lying close to zero flow. Since the oscillatory flow signal is subjected to significant distortion because of breathing flow transient at end inspiration, only end-expiratory R_{RS} values were retained.

2.5. Main protocol (12 rabbits)

One sequence of data acquisition consisted in 2–3 min recording including baseline, 30 s MC and recovery. Two MC were attempted at control and after Mch inhalation. At least 10 min were allowed to elapse between two acquisitions. It became apparent in the course of the study that AB's – here defined as a spontaneous increase in V_T of about 30% or more – were part of the ventilatory response to MC. In six of these animals, a graduated airtight glass syringe was connected to the airways through a side port inserted between the pneumotachograph and the FOT apparatus. During recovery, the airways were blocked and the lungs quickly inflated in expiration, to a volume similar to that observed during MC, so that each passive inflation was matched to the preceding AB.

2.6. Arterial blood gases (five rabbits)

An indwelling catheter was placed in one carotid artery. Arterial blood was sampled at baseline, during exercise and recovery. Blood gases and pH were measured using a blood gas analyser (ABL 330; Radiometer, Copenhagen, Denmark).