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The effect of body cooling on respiratory system mechanics and hysteresis in rats

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ABSTRACT

Literature reports and theoretical considerations suggest that body cooling may affect respiratory mechanics *in vivo*. To examine this hypothesis, healthy rats were studied using the end-inflation occlusion method under control conditions and after total body cooling. Respiratory mechanics parameters, hysteresis areas, the inspiratory work of breathing, and its elastic and resistive components, were calculated. After body cooling (mean rectal temperature from 36.6 ± 0.25 to 32.1 ± 0.26 °C), the ohmic and the additional visco-elastic respiratory system resistances, the hysteresis, the total inspiratory work of breathing, and its resistive components, were all increased. No significant changes were detected for the static and dynamic respiratory system elastance mean values, and the related elastic component of the work of breathing. These data indicate that body cooling increases the mechanical inspiratory work of breathing by increasing the resistive pressures dissipation. This effect is evident even for limited temperature variations, and it is suggested that it may occur in the event of accidental or therapeutic hypothermia.

1. Introduction

Literature reports strongly suggest that respiratory system mechanics may be dependent on body temperature variations (Rubini, 2011a). For example, rabbit airway smooth muscle cells exhibited increased isometric tension during methacholine challenge when exposed to cooling (Bratton et al., 1987), suggesting that airway mean diameter may be decreased, hence resistance increased, by temperature lowering. Furthermore, elastin fibres, which are known to be well represented in the alveolar walls, where exert a decisive role in influencing the pressure–volume characteristics of lungs, have been shown to exhibit a temperature-dependent stress–strain relationship, with increased stiffness as a consequence of cooling (Weinberg et al., 1995).

The alveolar surfactant content of the lungs tissues and lavage fluid has been described to decrease with temperature reduction (Kumar et al., 1980), suggesting that body temperature may affect lung hysteresis also. These possible temperature-dependent effects have not been extensively studied *in vivo* in the literature. A sole available paper described that total body warming induced a decrement in respiratory system hysteresis mean values in rats, although not significant (Rubini, 2011a). A hysteresis reduction with temperature increment was also observed in excised rabbit lungs (Lempert and Macklem, 1971). However, although indirectly, other data suggest that respiratory system hysteresis may exhibit temperature dependence, because of body warming or cooling influences on the biological activity of alveolar surfactant (Kumar et al., 1980; Bruni et al., 1996).

The possible consequences of temperature changes on respiratory system mechanics have been most of all investigated by means of cooling induction experiments in animals. For example, a reduction in lung static compliance has been reported in sheep subjected to total body cooling (Deal et al., 1970) and in positive-pressure ventilated excised rabbit lungs as an effect of temperature lowering (Nagao et al., 1977). Similar changes were also described in saline-filled excised dog lungs (Debes and Fung, 1992).

Animal experiment in dogs showed a cooling-induced airway resistance increment, either as an effect of low temperature-elicited reflex (Pisarri and Giesbrecht, 1997), or because of a directly induced activation of airway smooth muscle cells contraction (Salonen et al., 1991). Furthermore, local airway cooling, induced by cold air breathing, was shown to cause bronchoconstriction, either as a directly induced effect (Guleria et al., 1969) in humans, or as a consequence of vagally mediated reflex effect (Jammes et al., 1983; McFadden and Ingram, 1986). Asthmatic subjects seem to be particularly sensible to the effects of airway cooling (Horton and Chen, 1979; Sheppard et al., 1982; McFadden and Ingram, 1986; Eschenbacher et al., 1992; Kaminsky et al., 1995, 2000).

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However, few data are available describing the possible effects of total body cooling on various parameters of respiratory mechanics and hysteresis. Here are described experiments performed on the rat to investigate the *in vivo* effects of moderate and acute total body cooling on respiratory system mechanics and hysteresis. Respiratory mechanics parameters were measured by the end-inflation occlusion method, which has been previously extensively validated both in humans (Bates et al., 1985, 1988; D'Angelo et al., 1989; Coussa et al., 1993) and experimental animals (Reta et al., 2000; Peratoner et al., 2004; Rubini and Bondì, 2007; Rubini, 2010, 2011a,b; Rubini et al., 2011, 2012a), but has not been used to study the effects of body cooling. The method allows to measure, beside the respiratory system static and dynamic elastances, the resistance due to the frictional forces opposing the inspiratory flow in the airway (here termed "ohmic" resistance), and the additional visco-elastic resistance due to stress relaxation (Bates et al., 1985; Rubini, 2011a,b; Rubini et al., 2011, 2012a,b).

In addition, the *in vivo* effects of total body cooling on respiratory system hysteresis were also measured, and the consequences on the total work of breathing and on its elastic and resistive components quantified, by previously described methods (Coussa et al., 1993; Rubini, 2010, 2011a). On the basis of previously published data (see above), the working hypothesis was that even a modest body temperature reduction would acutely result in expected changes in respiratory mechanics parameters and in the related values of the total work of breathing and its components.

2. Materials and methods

The experiments were carried out on 10 Wistar albino rats of both sexes (mean weight 337 ± 32 g, 5 males). Additional 6 rats of similar characteristics served as control animals (see below).

The animals were housed and treated in accordance with the Italian law on animal experimentation (L. 116/92) and with the EU Directive 2010/63/EU for animal experimentation.

Rats were anaesthetized with 50 mg/100 g i.p. chloralose and then laid on a heated operating table. After a tracheostomy, a small polyethylene cannula (2 mm i.d., 5 cm long) was inserted through an incision in the second tracheal ring and firmly secured in place.

Positive pressure ventilation with a 10 ml/kg tidal volume and a 60/min breathing frequency (PEEP 3 cmH_2O) (Rodent Ventilator 7025, Basile, Italy) was begun, and was consistently maintained throughout the experiment.

Limb ECG probes were placed and the rats were paralyzed (vecuronium 2 mg/100 g i.p.).

Positive pressure ventilation was maintained for 5 min and respiratory mechanics were then measured using the end-inflation occlusion method (Bates et al., 1985, 1988; D'Angelo et al., 1989; Reta et al., 2000; Peratoner et al., 2004; Rubini and Bondì, 2007; Rubini, 2010, 2011a,b; Rubini et al., 2010, 2011, 2012a,b).

The ventilator was disconnected, PEEP was discontinued, and the tracheal cannula was connected to a constant flow pump (SP 2000 Series Syringe Pump sp210iw, World Precision Instruments, USA) set to deliver a tidal volume (VT) of 3 ml with a square wave flow (F) of 4 ml/s. The time for the rise and the fall of the flow was approximately 30 ms. The pump setting was carefully checked by directly taking measurements before beginning the experiments.

The lateral tracheal pressure proximal to the tracheal cannula was monitored (142 pc 01d, Honeywell, USA) and continuously recorded (1326 Econo Recorder, Biorad, Italy). Because abrupt changes in diameters were not present in the circuit, errors in flow resistance measurements, such as those reported by Chang and Mortola (1981), were avoided. The frequency response of the transducer and the pressure measuring system was tested by sinusoidal forcing and found to be flat up to 20 Hz. In accordance with the literature (D'Angelo et al., 1989; Reta et al., 2000), this frequency response was adequate to avoid mechanical artefacts in the pressure signal records.

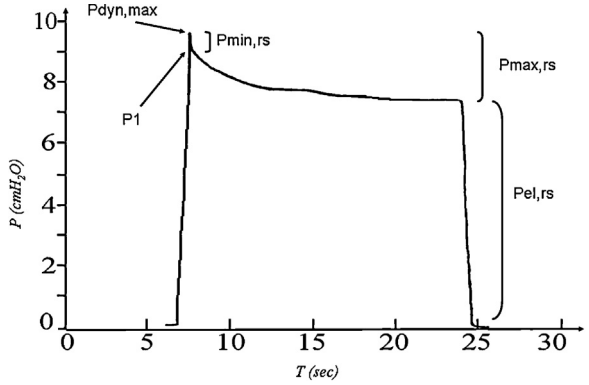

Fig. 1. Representative tracing of lateral tracheal pressure at flow interruption. The relevant pressures used for the calculations of respiratory system mechanics parameters are indicated: maximal pressure at end inflation (Pdyn,max), pressure immediately after flow interruption (P1), static elastic pressure of the respiratory system (Pel,rs), pressure drop due to the ohmic respiratory system resistance (Pmin,rs) and total pressure drop including the effects of stress-relaxation (Pmax,rs).

The end-inflation occlusion method was utilized to determine the parameters of respiratory mechanics: the static elastic pressure of the respiratory system (Pel,rs), the total resistive pressure drop (Pmax,rs) and the sudden resistive pressure drop at flow interruption (Pmin,rs) were measured on adequately magnified tracings (Fig. 1). Pmax,rs was calculated as the difference between the maximum value of pressure at end inflation (Pdyn,max) and Pel,rs. Pmin,rs was measured as the difference between Pdyn, max and P1, the pressure value immediately after flow interruption (Fig. 1).

To avoid a viscous pressure component in Pmin,rs, P1 values were identified by extrapolating the pressure tracings to the time the flow stopped. Thus, Pmin,rs represents the nearly instantaneous, Newtonian resistive pressure drop due to the "ohmic" resistance that theoretically occurs at infinite breathing frequency (Bates et al., 1987). Pmin,rs does not include the visco-elastic pressure drop that results from mechanical heterogeneity within the system and from stress relaxation. In contrast, the visco-elastic pressure drop is included in the Pmax,rs value (additional comments can be found in Section 4.1).

The mean pressure data obtained from 2 to 3 inflations for each rat were used to calculate the respiratory system static and dynamic elastances (Est,rs = Pel,rs/VT and Edyn,rs = P1/VT respectively) and the total resistance of the respiratory system (Rmax,rs = Pmax,rs/F). The total resistance value includes the "ohmic" inspiratory resistance to airflow offered by the airways and the resistance due to the movement of respiratory system tissues (Rmin,rs = Pmin,rs/F), and the visco-elastic resistance resulting from the effect of stress relaxation. This last component of Rmax,rs was isolated and quantified as "viscous" resistance (Rvisc,rs = Rmax,rs − Rmin,rs).

The "ohmic" component may be thought as the airway resistance occurring at infinite breathing frequency, when the time allowed for the pressure drop due to visco-elastic phenomena approaches zero. Instead, the additional visco-elastic component defines a higher resistance value which depends on a complete stress relaxation-linked pressure drop, theoretically occurring at zero breathing frequency. Accordingly, in real conditions *in vivo*, the actual resistance value will be a function of the breathing frequency, depending on the visco-elastic phenomena slow time course (D'Angelo et al., 1989).

The equipment resistance, including the tracheal cannula and the standard three-way stopcock, amounted to 0.0575 $cmH_2O/(ml/s)$ (Req) and was measured separately at a