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Modulation of defensive airway reflexes during continuous positive airway pressure in the rabbit

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ABSTRACT

Lung hyperinflation may alter the pattern of ventilatory reflexes in chronic respiratory disorders. The aim of the study was to test the effect of continuous positive airway pressure (CPAP) on ventilatory responses to mechanical stimulation of the trachea.

Twelve anesthetized, tracheotomized New Zealand rabbits were exposed to punctuate mechanical stimulation of trachea during control (0 cm H2O) and CPAP (5 cm H2O). The pattern of defensive responses allowed identification of cough reflex (CR) and expiration reflex (ER). Each type of response is expressed as percentage of the total number of stimulations.

CPAP was associated with marked alteration in the response to tracheal stimulation. CR was significantly decreased from 16.3% (12.5–19.3%) to 10.1% (4.2–14.6%); p = .03) during CPAP. The sequence of ER followed by CR however was up regulated during CPAP (31.2% (16.6–37.5%)), compared to control (12.5% (2.1–21.1%) p = .007).

CR inhibition with CPAP is consistent with reports in humans with chronic cough. The clinical relevance of up regulation of the sequence ER-CR requires further elucidation.

1. Introduction

Cough is a cardinal symptom in chronic respiratory disorders, and a vital reflex defense mechanism facilitating clearance of mucus, especially during exacerbations (Dougherty and Fahy, 2009; Nadel, 2013; Rosenfeld et al., 2013; Vilozni et al., 2014; Mccallion and De Soyza, 2017). Non-productive cough may also be observed in a variety of circumstances during activities involving the respiratory apparatus. For instance, it is a common experience in the pediatric lung function laboratory that repeated bouts of dry cough are observed after a spirometry in a child with cystic fibrosis. Moreover, cough-like expulsive efforts may be provoked by maximal lung emptying in subjects presenting symptoms of gastroesophageal reflux (Lavorini et al., 2011a; Lavorini et al., 2011b). Thus cough as a symptom may be triggered by changes in volume history. In addition, chronic cough has been shown to be associated with obstructive sleep apnea syndrome, with the increasing evidence that continuous positive airway pressure (CPAP) during sleep is associated with significant resolution of the symptom in the long term (Sundar and Daly, 2011; Wang et al., 2013; Sundar and Daly, 2014).

The role of absolute lung volume in controlling the reflex of cough is especially relevant to subjects undergoing nighttime positive airway pressure breathing and those with chronic airway disease. Hyperinflation of the lungs frequently occurs in the progression of the disease, and expiratory flow limitation during tidal breathing may result in inadvertent positive end expiratory pressure and dynamic elevation of the functional residual capacity (Gibson, 1996), possibly altering the pattern of ventilatory reflexes. Data on defensive responses elicited by stimulation of the respiratory tree in relation with lung volume in humans are limited. CPAP was reported not to alter the pattern of ventilatory responses to intra tracheal administration of distilled water in anesthetized adult females (Nishino et al., 1989). However, with mechanical stimulation of the airways, animals were reported to exhibit down regulation of cough responses by lung inflation (Tatar et al., 2008) or weakening of the inspiratory and expiratory components of cough (Javorka et al., 1994). In the latter experiments

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however, it was not specified whether the stimulus was applied in inspiration or expiration, while it has been shown that the timing within the breathing cycle is important in determining the type of response (Varechova et al., 2010).

It was thought that applying single punctate mechanical stimuli to the trachea in a well characterized animal model (Varechova et al., 2010; Varechova et al., 2012; Coutier-Marie et al., 2017) would help delineate characteristics of these defensive reflex responses triggered at different times of the breathing cycle, when the functional residual capacity has been elevated. The aim of the study was therefore to test the effect of CPAP on ventilatory responses to mechanical stimulation of the trachea. The main end point was the frequency and intensity of triggered defensive responses.

2. Material and methods

2.1. Animals

Experiments were performed in 12 New Zealand adult rabbits (2.9 ± 0.3 kg). Animal experiments carried out in 2012 were in conformity with the European Communities Council (ECC) guidelines for animal care procedures and the French legislation application of the ECC directive 86/609/EEC (decree number 87–848). The study was conducted under the license (A54518-03409) from the French ministry of agriculture and fisheries (Ministère de l'Agriculture et de la Pêche) and the Ministry of higher education and research (Ministère del'Enseignement Supérieur et de la Recherche; A54518-03409) and supervision by the regional veterinary services (Services Vétérinaires Départementaux de Meurthe et Moselle). Ethics committee approval was not required, in accordance with French legislation in place at the time of the study.

2.2. Anesthesia, analgesia, and euthanasia

The induction of surgical anesthesia was performed with a mixture of urethane (500 mg/kg), alpha-chloralose (50 mg/kg) in 5 animals and with pentobarbital (30 mg/kg) (Pentobarbital sodique, Ceva Santé Animale, France) in 7 animals. Initial and supplemental doses (given every 2 h) were injected through the ear vein. Preoperative local analgesia before tracheotomy was performed by subcutaneous administration of Laocaine 20 mg/ml (Intervet, Schering-Plough Animal Health, France). After induction, the depth of anesthesia and analgesia was assessed every 20 min by testing the pedal withdrawal reflex (foot pad pinch on both hind feet) and respiratory rate. In the case of the animal responsiveness to painful stimuli and/or increase in respiratory rate, the animal was supplied with additional anesthesia and re-tested before continuing experimental procedures. Euthanasia was achieved with intravenous administration of 5 mL of Doléthal (Vétoquinol S.A., France).

2.3. Animal preparation

The anesthetized animal was placed in the supine position. Rectal temperature was continuously monitored with an electrical thermistor (Physitemp Instruments, YSI 402 Clifton, NJ USA) and maintained at 38 °C using circulating warm water pad. The electromyogram of the rectus abdominis muscle was performed by insertion of bipolar insulated fine-wire electrodes according to Basmajian and Stecko (Basmajian and Stecko, 1963) to further characterize the active expiration of cough and expiration reflex. An upper cervical tracheotomy allowed the insertion of a tracheal cannula that was connected to a pneumotachograph (No. 0 Fleisch pneumotachograph with linear range ± 250 mL/s) and to the mechanical stimulation apparatus. The pneumotachograph was calibrated before each experiment using a 20 mL calibration syringe.

2.4. Constant positive airway pressure

Continuous positive airway pressure of 5 cm H2O, most widely used in small experimental animals (Miller et al., 2004; Xue et al., 2011; Reyburn et al., 2016; Ricci et al., 2017), was delivered by a CPAP system (SULLIVAN® V ELITE (Res Med ltd, Sydney, Australia) that was connected in series to the pneumotachograph. At least 10 min were allowed for a stable state to be reached, defined as tidal volume of 25–30 mL and respiratory frequency of 17–20 breaths/min.

2.5. Mechanical stimulation of trachea

The mechanical stimulation of the trachea (at the level of the carina) was performed using a semi-rigid wire (nylon fiber) rotated by a23HSX-102 universal motor (Rare EarthMagnet Stepper Motors ref. 23HSX-102, Mclennan Servo Supplies Ltd., Ash Vale, UK) that achieved a square wave stimulus automatically triggered 50 ms from the beginning of inspiration or expiration. The beginning of inspiration or expiration was detected electronically as the flow signal reached a positive or negative value, respectively. The stimulation time could be varied in a stepwise manner between 50 and 600 ms. Stimulation was performed when breathing was regular.

The stimulus pattern to be used throughout in each animal was determined as the minimal duration able to elicit 4 positive responses (cough and/or expiration reflexes) out of 4 trials (2 in inspiration and 2 in expiration). Based on our prior experience, the initial duration set at 100 ms, and depending on the response observed, altered stepwise − down to 50 ms or up to 600 ms (according to the protocol detailed in Fig. 1).

This stimulus duration was then applied 20 times during control (C) and 20 times during CPAP in each animal. At least 1 min was allowed to elapse between two mechanical stimulations. The signals of airflow, integrated volume, EMG and stimulus were fed to the LabChart recorder (ADinstruments, Oxford, UK), digitized at 1000 Hz and used for on-line and off-line analysis.

2.6. Data analysis

2.6.1. Ventilatory responses to tracheal stimulation

The defensive responses were identified from the change in tidal volume (VT), peak expiratory flow (V'Epeak) and rectus abdominis EMG (Varechova et al., 2012).

Cough reflex was defined as an increase of VT followed by an increased V'Epeak associated with a burst of activity in the rectus abdominis EMG. Expiration reflex was defined as a brief V'Epeak of variable amplitude associated with burst of activity in the rectus abdominis EMG, without prior increase in VT.

In order to take into account the spontaneous between-breath variability, an unbiased differentiation of cough from expiration reflex was achieved by a statistical evaluation of VT between stimulation and reference breath. Tidal volume of reference breath was determined as the mean of 3 breaths prior to stimulation and its upper limit as mean + 3 standard deviations. The cough reflex was identified when VT of stimulation breath was higher than upper limit of reference VT. Multiple defensive responses consisted of a bout of several cough reflexes (CR), a bout of several expiration reflexes (ER) or a bout of coughs preceded by expiration reflexes (ERCR). Multiple defensive response consisting from a bout of cough(s) followed expiration reflex(es) was elicited exceptionally ($< 1\%$ of the total number of stimulations) and counted as CR.

2.6.2. Statistical analysis

Statistical analysis was performed using the SYSTAT 13 package (San Jose, CA, USA). Following quantitative parameters were analyzed for each animal: