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Effect of airway remodeling and hyperresponsiveness on complexity of breathing pattern in rat

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ABSTRACT

The complexity of respiratory dynamics is decreased, in association with disease severity, in patients with asthma. However, the pathophysiological basis of decreased complexity of breathing pattern in asthma is not clear. In the present study, we investigated the effect of airway remodeling and hyperresponsiveness induced by repeated bronchoconstriction (using methacholine) on breathing pattern in rats with or without allergen-induced sensitization. Entropy analysis of respiratory variability showed decreased irregularity (less complexity) of respiratory rhythm in this rat model of asthma. Airway remodeling and hyperresponsiveness induced by repeated bronchoconstriction also led to increased regularity of respiratory dynamics in sensitized rats. However, these airway alterations had no significant effect on the complexity of breathing pattern in non-sensitized rats. Our results indicate that mechanical respiratory alterations cannot per se attenuate the complexity of respiratory dynamics, unless there is an underlying inflammation. We suggest further studies on underlying mechanisms of breathing variability with focus on respiratory control alterations due to airway inflammation.

1. Introduction

Normal human breathing reveals a complex pattern related to an intricate network of nonlinear interactions and feedbacks (Thamrin et al., 2016). Through these dynamical processes, both respiratory rhythm and volume fluctuate continuously under a delicate equilibrium to maintain adaptability to external or internal stimuli (Raoufy et al., 2016). In this context, a healthy respiratory system is adaptive and fluctuates normally. Chronic lung diseases, especially asthma, have variable clinical symptoms and represent complex behavior which may be associated with a shift in dynamics of respiratory system toward either too regular or too irregular (Raoufy et al., 2016).

Analyzing such nonlinear fluctuations may not only improve traditional assessments in diagnosing the onset of illness, its severity and prognosis (Thamrin et al., 2016; Kaminsky et al., 2017), but also could provide new insights into the different pathophysiological characteristics of respiratory diseases, such as asthma (Raoufy et al., 2016). For instance, previous studies demonstrated that decreased irregularity of airflow pattern in asthmatic patients is associated with increased severity of airway obstruction (Veiga et al., 2011), and decreased fractal-like correlations of peak expiratory flow fluctuations over long term may be suggestive of poorly controlled asthma (Frey et al., 2005). The results of our recent study also showed a reduction in the long range correlation, irregularity and chaotic nature of breathing pattern in asthmatic patients, particularly in uncontrolled state (Raoufy et al., 2016). Therefore, complexity analysis of respiratory dynamics may represent a novel physiologic marker to facilitate diagnosis and clinical assessment of respiratory disorders (Raoufy et al., 2017).

Understanding the basis of such complex behavior is of physiological interest and can make new pathophysiological insights into the characteristics of illnesses, and hence may provide opportunities to develop better-targeted intervention and treatment strategies (Thamrin et al., 2016; Raoufy et al., 2016). The pathophysiological basis of respiratory pattern decomplexification in asthma is not clear and the underlying mechanism of this phenomenon can be investigated further by studies on experimental models of asthma. However, despite the growing interest in the nonlinear dynamics of respiration (Peng et al., 2002), to our knowledge, there is no study investigating the complexity of breathing pattern in animal model of asthma.

Numerous potential sources from micro- to macroscopic scales may be responsible for the fluctuations in the respiratory pattern (Thamrin et al., 2016). Asthma is associated with airway remodeling and hyperresponsiveness, and some alterations in respiratory mechanics may be involved in a shift in the dynamics of respiratory system away from the normal fluctuation in asthma. In a recent study, we presented a new animal model for development of airway remodeling and hyperresponsiveness, without allergic inflammation, induced by repeated bronchoconstriction in rats (Eslami-Behroozi et al., 2017), which can be

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used for studying the effect of airway remodeling and hyperresponsiveness on complexity of breathing pattern.

Since asthma is associated with airway remodeling and hyperresponsiveness, our hypothesis was that such alterations in respiratory mechanics may be involved in the decomplexification of respiratory pattern in asthma. In the present study, we compared respiratory interbreath interval (IBI) as well as respiratory volume (RV) fluctuations among controls and asthmatic rats, and also investigated the effect of airway remodeling and hyperresponsiveness on breathing pattern in rats with or without sensitization.

2. Methods

2.1. Experimental groups

Specific pathogen-free male Sprague-Dawley rats, weighting 250 ± 20 g, were randomly divided into five groups, as previously described (Eslami-Behroozi et al., 2017). Briefly, animals received two intraperitoneal (i.p.) injections of the following agents on Day 0 and Day 7, and eight inhalations of aerosols using a compressor nebulizer (Pari, Starnberg, Germany) for 30 min, every 2 days from Day 14 to Day 28. *Saline group*, animals received and inhaled saline during the whole experiment; *Ovalbumin (OVA) group*, 2 i.p. doses of OVA–Al(OH)3 [1 mg OVA (Grade III; Sigma) and 200 mg Al(OH)3, in 0.5 ml saline] and 8 inhalations of OVA (1 wt%/vol); *MCh group*, 2 i.p. doses of MCh (16 lg/ml, Provocholine, Canada) and 8 inhalations of MCh (2 mg/ml); *OVA–saline and OVA–MCh groups*, 2 i.p. doses of OVA–Al(OH)3, 3 inhalations of OVA, to induce an airway remodeling (Sapienza et al., 1991), and then 5 inhalations of saline or MCh.

All of the protocols were approved by the "Ethical Committee of Faculty of Medical Sciences, Tarbiat Modares University".

2.2. Recording of respiration

The respiration of conscious rats was recorded on Day 0 (before i.p. injection) and 24 h after the last aerosol exposure, using a custom-built whole-body plethysmograph (Kabir et al., 2010). This consisted of an animal chamber made of transparent Plexiglas cylinder (i.d. 75 mm, length 300 mm, volume 1.32 L, and wall thickness 5 mm) with oppositely aligned inlet and outlet ports in each wall. Room air was continuously pumped into the chamber at a controlled flow rate (4 L/min) in order to prevent any rise of CO2 in the plethysmograph. A plastic Y-connector was placed at a distance of 10 cm from the chamber's outlet, and one of the exit ports was linked to one input of a differential pressure transducer (MPXV7002DP, Freescale Semiconductor Inc.), the other input being exposed to the room air. The plethysmograph chamber was also connected to one input of a CO2 sensor (MG811, Sandbox Electronics Inc.), by mean of polyethylene tubing linked to second exit port of the Y-connector.

In order to allow the animals to get acclimated to recording chamber, another transparent plexiglas cylinder of the same size as the plethysmograph chamber was placed in all home cages. In addition, animals were placed to recording box one hour per day for 7 days prior to recoding respiration. On the time of experiment, animals were gently guided into the chamber, without being forced. Each experiment consisted of almost 10 min initial recording period (as the acclimatization time) which was then followed by a 60-min main recording period.

2.3. Breathing pattern analysis

The respiratory signals were digitized at a 10 KHz sampling rate and acquired using a PowerLab A/D converter (ADInstruments, Sydney, Australia) (Fig. 1a). We were observing the animals during the recording in order to recognize the artifacts due to animals' movements, sniffing, *etc*. Twenty minutes of data with minimum artifact was selected for breathing pattern analysis. The peaks of each respiratory signal were detected and visually verified, and then the peak-to-peak intervals and the amplitude of peaks were considered as the IBI and RV series, respectively (Fig. 1). All time-series were normalized to have a mean of zero and standard deviation = 1, in order to allow comparisons of data sets with different degrees of variability.

Complexity of breathing pattern was quantified by calculating sample entropy (SampEn), which is the negative natural logarithm of the conditional probability that epochs within a physiological-series that match within tolerance r for m points will also match for $m + 1$ points (Richman and Moorman, 2000). In our analysis, we computed SampEn of IBI and RV series by the values of 2 for m and 0.2 for r, using MATLAB code available from the physionet (http://www.physionet.org). We also used multiscale entropy (MSE) to quantify the degree of irregularity in each series over multiple time scales (Costa et al., 2002).

2.4. Measurements of airway responsiveness

As previously descripted (Eslami-Behroozi et al., 2017), 24 h after the last aerosol exposure, animals were anaesthetized with intraperitoneal injection of urethane (1.5 g/kg) and then they were subjected to tracheostomy and mechanical ventilation with a volume controlled ventilator (Harvard Apparatus, Holliston, MA) at a tidal volume of 1.0 ml/100 g and a rate of 70 breaths/min. Afterwards, animals were paralyzed by intraperitoneal injection of pancuronium bromide (0.2 mg/kg). Pulse oximetry was continuously monitored.

For assessment of airway responsiveness to cumulative doses of MCh, increasing concentrations (0.25–0.5–1–2–4–8 mg/ml in saline) of MCh were inhaled for 60 s at 5-min intervals. The airway responsiveness was expressed as the percentage of airway pressure provokes by MCh to basal pressure.

2.5. Histology, immunohistofluorescence, and morphometry

Medium size bronchioles in lung sections were blindly evaluated for morphological alterations and inflammatory cell infiltration, as previously described (Eslami-Behroozi et al., 2017). Briefly, the lung sections were stained with hematoxylin and eosin (H & E) to identify the airway inflammatory cell infiltration, periodic acid Schiff (PAS) to detect mucus producing goblet cells, and Masson-trichrome to determine subepithelial collagen deposition. For detection of α-smooth muscle actin (α-SMA), the lung sections were incubated with the primary antibody against α-SMA (Abcam, at 1:100 dilution, ab5694), and then with fluorescent-conjugated secondary antibodies (goat anti-rabbit IgG-FITC, 1:200, SC-2012). The expression of α-SMA was estimated by measurement of fluorescence intensity.

2.6. Statistical analysis

The GraphPad Prism V6.0 (GraphPad Software, San Diego, CA) was used for statistical analysis of data. The differences in histological and breathing pattern among groups were assessed by a one-way ANOVA with a Bonferroni post-test or a Kruskal–Wallis non-parametric test with a Dunn's post-test. Changes in airway pressure and MSE for groups were compared using 2-way ANOVA, with the Bonferroni post hoc test. All results are presented as mean ± standard error of the mean (SEM). P-values less than 0.05 were considered statistically significant.

3. Results

3.1. Respiratory dynamics in asthmatic rats

The sensitized rats challenged with allergen (OVA group) had significantly higher levels of airway inflammation ($p < 0.001$) (Fig. 2c), α-SMA expression ($p < 0.001$) (Fig. 2d), subepithelial collagen-band thickness ($p < 0.001$) (data not shown), and the number of goblet cells ($p < 0.001$) (data not shown) as well as AHR ($p < 0.001$) (Fig. 2b)