Detection of Airway Partitioning Following Unilateral Nasal Stimulations by the Forced Oscillation Technique in Rats

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Abstract- Nasal mucosa has an extraordinary nerve supply with unique geometry that encompasses complex physiology. Among these, side-specific predilections to the respiratory and autonomic centers are the interesting issues that have been raised about the consequences of the nasal irritations. The aim of the study was an evaluation of how intranasal stimulation influences lung mechanics and determines whether unilateral stimulation produces side-specific partitioning responses. Tracheotomized-paralyzed rats received unilateral air-puff stimulation. Inspiratory pressure- volume (P-V) curve was obtained. Low frequency forced oscillation technique (FOT) was used to detect changes in central and peripheral airways. Mean airway pressure significantly increased to >10 cmH_2O in the presence of $5cmH_2O$ of positive end-expiratory pressure. Elastance was significantly changed, and significant higher airway resistance (Raw) and lower reactance (Xrs) were noticed in peripheral airways following different side of stimulation. Calculated inspiratory P-V curve showed significant deviations in transitional, rising and maximal pressures following stimulations. Transitional left-side shifting was observed following right side stimulation, whereas left side stimulation shifted the curve to the right. May be altered respiratory mechanics is the consequences of bimodal pressure-volume relationships observed in central and peripheral airways following nasal stimulation.

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Introduction

Nasal surgeries and air pollutions nowadays are modern life sequels that invoke irritant stress on the nasal mucosa due to altered energy dissipation and direct chemical contaminations (1-4). A wide-variety of sensory innervations inside nasal cavities and co-existence of different receptors in the nasal mucosa are well documented. It has also been reported, that this area has multiple regulatory roles on various physiological contexts other than air-conditioning (5-7). Neural labeling of expressing c-fos immunoreactive trigeminal-associated nuclei inside brainstem, also further support the target site of nasal stimulations (8-11). These studies consecutively showed the origin pathway (trigeminal afferents) and effector arm (vagus nerve) of the reflex (12-15).

Most of responses elicited by upper airways irritations are autonomic responses in nature. Among these, autonomic side-dominancy is one of the most interesting criteria in which that response raised from manipulations in one nostril, had been different to that of contralateral one (16-19). Corresponded with this issue, are cerebral hemispheric dominancy and contradictory responses elicited in the cardiorespiratory parameters which are already stated during yoga exercises (16,20,21). Considering the pattern of breathing and overall changes in resistance and elastance, several investigations addressed different outcomes attributed to the site and mechanism of stimulations (1,22-24). Application of different nasal irritations with nylon fiber, nasal pads, saline and capsaicin installation, cold air, air-jet and air-

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puff stimulations also have been shown to elicit multiple cardiorespiratory responses (6,13,25,26). What is obvious from previous studies, respiratory system mechanics is readily affected during the onset of nasal stimulation. Primarily, it occurs because of its immediate effect on ventilatory effort and modulation of bronchomotor pathways (27-30). According to the side-specific diversity, it may intuitively flash on the mind whether unilateral nasal stimulation also could establish non-uniform change(s) in the respiratory system? Another assumption is that whether possible resulted responses in the central (large) airways are same as to the responses in the peripheral ones (small airways)? We have implemented the Forced Oscillation Technique (FOT), to address the partitioning of central and peripheral airways, for the inherent accurate estimation capability being inside the context of the impedance spectrum.

Materials and Methods

Study design

Design of the present study was constructed primarily under the guidelines of Institutional Review Board Ethics of Tehran University of Medical Sciences regarding animal care and use, and received respective approval. Thirty Wistar rats (180-230grs) used for assessments of respiratory mechanics following nasal air-puff stimulation. Animals were randomly divided to the control and two main unilateral stimulation groups consist of right and left side nasal air-puff stimulation subgroups (n=6). Airway pressure and flow were monitored in anesthetized animals, and respiratory mechanics was estimated based on FOT data for detecting airway partitioning and respiratory system compliance curves.

Animal preparations

The animals were anesthetized by intraperitoneal dose of ketamine hydrochloride (65 mg/kg) and xylazine (2.5mg/kg). Adequate anesthesia assured with corneal and pedal reflexes. Femoral vein was cannulated for drug delivery (atracurium 30μg/kg/h) and tracheostomy was performed. A tracheal polystyrene cannula (ID =2.5mm) was inserted into the distal trachea and animal fixed in the supine position on warming pad. Mechanical ventilation was provided by a conventional belt-derived ventilator (Palmer-England) with a tidal volume (VT) of 2.6 ml/kg with 12 cycles/min revolutions.

Nasal air-puff stimulation

Before the start of stimulation, laryngeal nerves were sectioned bilaterally with a glass hook. Pressure-controlled air-puff stimulation (12-15cmH2O, 5L/min, 25/min) was delivered continuously with a costume designed a conventional respirator (room air, 23°C) for 60 min ipsilaterally through a polyethylene catheter (ID: 1.3mm), 5mm beyond the nostril opening (31). Contralateral nostril left intact, and the intrinsic activity of larynx as the effectiveness of stimulation was confirmed.

Forced oscillation technique

The FOT measurement used in this study was employed as described previously (32,33). Briefly, a multi-frequency (0.2-8.1Hz) flow waveform signal was applied at the airway opening under the inductive signals from digital signal source inside Simulink platform to the loudspeaker (34). The measurements were conducted on inspiratory tidal flow in paralyzed/ ventilated animals. Two pressure levels assigned to the oscillation amplitudes inside the wave tube. Transducers for airway opening pressure (Pao)(±100 cmH_2O, Capto-SP844- USA) and airflow (V')(0-5L/min FSG4003, Siargo, China) were placed in-line, and data recordings were performed using the chart recorder (Lab Chart v5, AD Instruments- Australia) and then, filtered for frequency isolation. Fourier analysis performed on ensemble Hanning averaged signals over time of 30s and calculated the impedance of the respiratory system from the estimation of power spectral density for each applied frequency. Then calculated impedance is partitioned to the real part; resistance (R), and imaginary part; reactance (Xc). A coherence function is also obtained at each frequency investigated in order to evaluate the interdependency of pressure to flow.

Measurement of respiratory mechanics

Measurements were performed on anaesthetized, tracheotomized rats. Animals breathed room air via a Y-tube for prevention of mixing inspired air with expired gas (dead space=0.2 ml) (35). Once connected to a ventilator, different PEEP levels ranging from 0-15cmH_2O, introduced with same ventilation settings (I: E=1) for detecting minimal significant pressure excursion. Periodical sighs applied in the respiratory tract below mean airway pressure of 10 cmH_2O. Mean ±SEM of inspiratory P-V curve was calculated from the stepwise changes in thoracic gas volume (0.2 ml/s). Peak inspiratory pressure (PIP) was plotted against time for measuring of within-breath observation of respiratory compliance in closed-loop preparation. Mean airway pressure also calculated from mean root square (RMS) of pressure for better elucidation of time-dependent changes following stimulation on static properties of the lungs. Macroscopic changes in total resistance (R) and inertance (I) were measured to determine airway is narrowing and/or collapse. Total elastance (E) and upper inflection pressure point (UIP) were also measured to evaluate the elastic recoil and development of intrinsic PEEP, respectively. Respiratory system impedance (Zrs) was measured according to the method introduced by Kaczka *et al.,* (32) against a broadband frequencies ranging from 0.2 to 8.1Hz. Two pressure and flow amplitude was selected for each component as external force acting upon the respiratory system for detecting related significant wave excitation. Real (Newtonian airway resistance (Raw)) and imaginary (capacitive reactance (Xc)) were estimated from the Zrs as a function of frequency. Non-linear regression analysis of quasi-static compliance (Cqs) was calculated from Xc, ($Cqs= 1/\omega kXc\ (dv/dt)$, ($\omega=2\pi fk$)) as the pressure changed per unit volume fluctuation and then fitted on obtained compliance data (36,37).

Statistical analysis

One-way ANOVA was used to compare baseline variables at different PEEP levels. Repeated measure ANOVA procedure followed by step down Bonferroni t-test were used to pairwise comparison of mechanical parameters after multiple treatments at different PEEPs. Estimation of sample mean±SEM of the data are presented, and the significance level accepted at $P<0.05$. Functional data analysis on prediction of polynomial fitting formula was performed by F test on the curves obtained from three-parameter sigmoidal fitting of discrete data points. Statistical analyses were performed with Sigma-Stat statistical computer package (Sigmaplot version 11.0.0.7).

Results

Figure 1a shows mean values of peak inspiratory airway-opening pressure (PIP) from baseline to the end of the study. After onset of the stimulation, PIP significantly increased to 13.3 and 16.9cmH2O, in the right side (RS) and left side (LS) stimulation groups, respectively in the presence of PEEP=5cmH2O. Thereafter, values of the stimulation groups rose to 14.4 and 19.7 cmH2O, respectively ($p<0.01$). Similarly, mean airway-opening pressure (Figure 1b) of RS and LS groups increased to 9.4 and 11.3cmH2O, respectively ($p<0.05$), although transitional significant decrease to 6.6 cmH2O was seen ($p<0.05$) when PEEP was withdrawn at the onset of the study protocol.

Figure 2a, shows the mean value of airway resistance from control and different stimulation groups. Resistance value stacked according to the amplitude of airflow (lower and higher mean amplitude, respectively). Baseline Raw represents primarily the resistance calculated from 5Hz oscillation, which was not significantly different from traditional resistance. Ten minutes after RS and LS stimulations, Raw gradually increased and reached the level of statistical significance at 5Hz. calculated resistance following stimulation of both cavities did not show significant changes as compared to the control group. As depicted in figure 2b, changes in inertive properties of the respiratory system, did not show any significant differences between investigated control and stimulation groups.

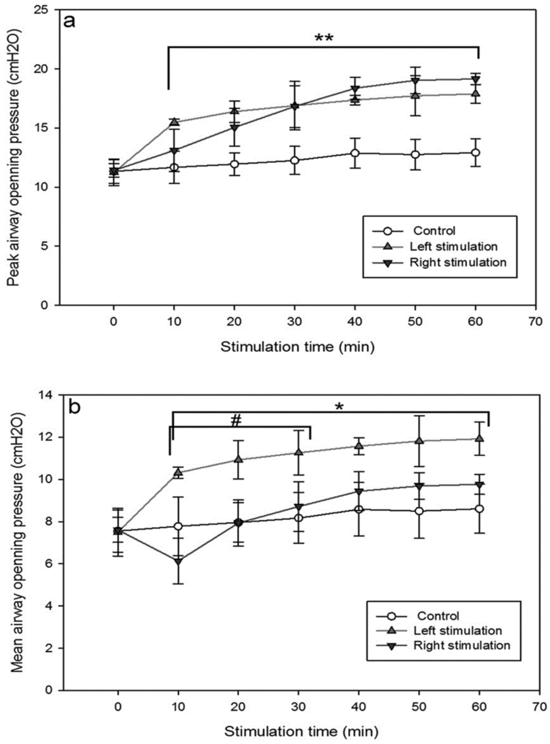

Figure 1. Time course of peak (a) and mean airway opening pressure (b) in control, RS and LS stimulations from baseline. Data of each group are means ±SEM of n=6 rats, ** indicates a significant change when compared with the control group ($P<0.01$), # indicates a significant difference between two stimulation groups at $P<0.05$

Macroscopic changes in elastance (E) revealed considerable and significant difference after RS stimulation as compared to the control (Figure 2c). There were no significant differences in E after LS and/or stimulation of both cavities. The changes of E was greatest at higher PEEP levels, although the data shown here are averaged E over the range between 5-

15cmH2O. In figure 2d, changes in inspiratory upper inflection pressure point is depicted. As shown, LS stimulation resulted in a significant increase in UIP as compared to control the group. Despite an observed a rising trend, RS stimulations did not result in a significant difference.

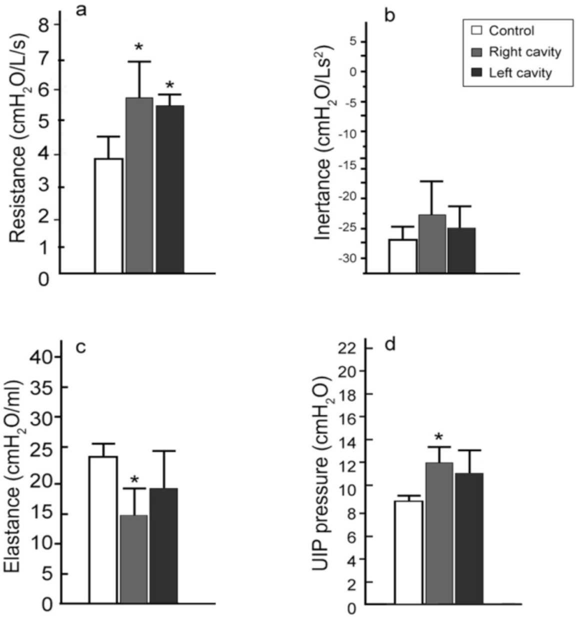

Figure 2. Effect of intranasal mechanical stimulation on total respiratory resistance (a), inertance (b), elastance (c) and upper inflection point pressure (UIP) (d) in anesthetized/paralyzed rats. Data are expressed as mean±SEM of n=6 rats. * indicates a significant difference when compared with the control group ($P<0.05$)

As depicted in the table 1, significant changes are easily observed from the effect of nasal stimulation to that of the control group. Mean value of R was systematically higher when the pressure amplitude was increased, and higher pressure levels are consistent with higher flow amplitudes. Overall progression of R from 0.045 to the 1.194 after LS stimulation was significantly different from the control group in high frequency component (8.1Hz). Furthermore, a significant difference was observed in comparison between LS and RS in 8Hz. RS stimulation did not show a significant difference as compared to control the group.

Figure 3 shows the mean Rrs and Xrs curves in control and different groups of stimulations. Primarily, frequency dependence of the Rrs and Xrs was observed. Stimulation elevated the values for Rrs and increased the frequency dependency, the behavior in which systematical identifiable response between RS and LS stimulation are prominent. Reactive property of the respiratory system also exhibit similar characteristics; Xrs significantly changed over the bandwidth with increased frequency dependence, specifically at the lower components and an increase in resonant frequency (probable zero line intercept).

Figure 4 shows the mean value of volume measured at different pressure levels between 0 and 15cmH2O. Inflation factors at pressure of 3, 5, 7, 10 and 15 cmH2O were 1.05±0.04, 1.02±0.03, 1.07±0.04, 1.08±0.07, and 1.03±0.06, respectively. No significant relationship was found between pressure and the inflation factor, and order of the pressure applied too. Greatest volume change was 5.6±3.2% at pressure of 5.7cmH2O. Nasal stimulations showed significant deviation in inspiratory P-V curve as compared to control the group. Left side (LS) stimulation significantly shifted the curve toward the right with lowering maximal volume excursion. Right side (RS) stimulation in contrast, significantly shifted the inspiratory P-V curve toward the left but with almost the same lower volume excursion (Figure 4). So there was no significant difference in maximal pressure –volume between RS and LS, however significant difference was observed between RS and LS at transitional and rising phases ($P<0.01$).

Table 1. Pulmonary resistance at two pressure amplitudes obtained from different excitation frequencies in ventilated rats, with right and left side stimulations

Frequency (Hz)	Pressure amplitude (cmH$_2$O)	Flow amplitude (ml/s)		R$_{aw}$ (cmH$_2$O/L/s)		
				Control	RS	LS
0.2	2	9		0.137*	0.069	0.045
	5	16		0.167*	0.101	0.088
1.85	2	11	18	0.143	0.073	0.070
	5			0.184	0.094	0.091
3.61	2	12	20	0.045	0.087	0.075
	5			0.082	0.116	0.117
8.1	2	13		0.071	0.094	1.352*** †
	5	15		0.074	0.153	1.194*** †

*&*** indicate a significant change when compared with the control group at $P<0.05$ and $P<0.001$, respectively. † indicates a significant difference between two stimulation groups at $P<0.05$

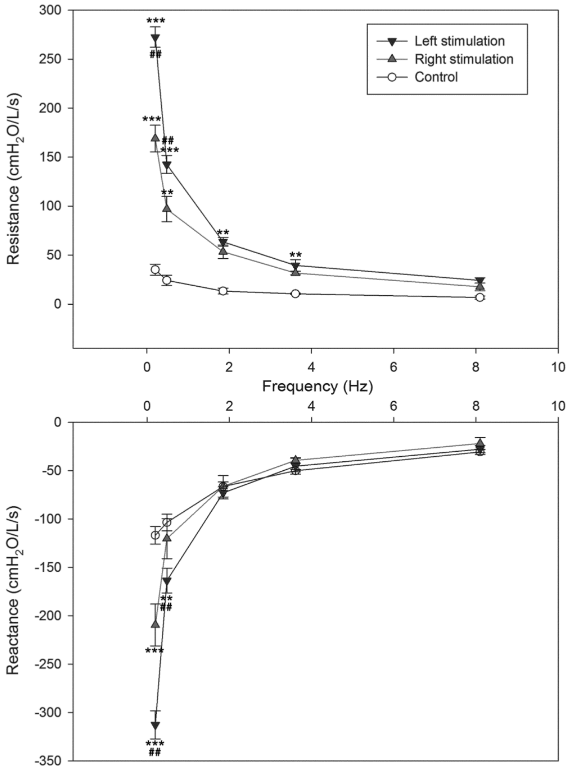

Figure 3. Comparison of impedance spectra and frequency dependence of mean ±SEM values of respiratory system resistance (top) and reactance (bottom) as a function of frequency in control and different stimulation groups. **&*** indicate a significant change when compared with the control group ($p<0.01$ and $p<0.001$, respectively). ## indicates a significant difference between two stimulation groups at $p<0.01$

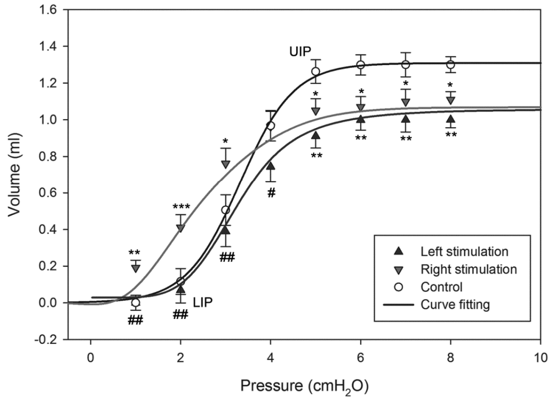

Figure 4. Quasi-static inspiratory pressure-volume curves of control rat at baseline and following nasal stimulation groups. Data shown are mean ±SEM of n=6 rats. * indicates a data point with a significant difference when compared with the control group ($p<0.05$), #&## indicate significant differences between two stimulation groups at $p<0.05$ and $p<0.01$, respectively. LIP: lower inflection point, UIP: upper inflection point

Discussion

To elucidate the mechanical changes of the respiratory system due to unilateral nasal mechanical stimulation, we investigated the forced oscillation method. Our results showed that two major findings are intuitively perceived from these results. First, application of nasal air-puff stimulation resulted in side-specific alterations in mechanical constituents. Second, alteration in quasi-static P-V curves can be readily attributed to the non-uniform behavior of the bronchial tree in response to an uniform manipulation.

Our previous study on respiratory mechanics in spontaneously breathing rats showed that changes in the impedance and elasticity are changed as a function of respiratory rate, after nasal stimulation(1). In previous investigations also, cold air stimulation directly have resulted in a reflex increase in airway resistance (Raw) in normal subjects, or mainly those with bronchial hypersensitivity (38,39). Kaufman *et al.*, reported an immediate increase in Raw following nasal packing with a gauze pad (40). Fontanari *et al.,* and Ishizuka and Usui also reported a bronchoconstriction response after nasal packing (26,41). Meanwhile other investigations showed a less uniform responses, and sometimes controversial. For example, Tomori and Widdicombe showed a rapid adapting dilatory response after nasal irritation with a nylon fiber in cats (42). Studies on the effect of conditioned continuous air-jet or intermittent air-puff stimulation also left similar inconsistencies behind (9,17,43). Pranayamic breathing (a type of Yoga Exercise) exhibited side-specific changes in cardiorespiratory modalities following alternate nostril breathing (16,20,21,44). It is postulated that complex innervations of the nasal area, concomitant with hemispheric and autonomic dominancy might be responsible for such phenomena (22,23,45,46). Going on in this section, more details in this topic will be discussed.

In the present study non-significant changes in inertance indicate that, there were no considerable changes in air-mass movement and gas acceleration inside the airways. However, this does not exclusively throw down the fact of airway narrowing. Data obtained from measurement of PIP and MIP showed an increased airway pressure due to stimulation, though significant differences were observed in between-group analyses.

Increased E is another corroborating fundamental which supports our statement of airway narrowing, because of increased tethering tension as a result of airway's smooth muscle contraction. In an asymmetrical P-V curve, because of the shape of the airway's elasticity curve, the airways are susceptible to narrow under the influence of collapsing pressure, than to distend for an equivalent increase in internal pressure (Figure 4) (47,48). It is this asymmetry which resulted in the marked frequency dependence of Rrs. It has been shown that, when airway narrowing increased, Rrs and Xrs exhibit sharper alterations (48). Similarly, resistance and reactance presented considerable shifts, mainly in the lower band which lasted afterward. Our results were in close agreement with supporting physiological fundamentals, previously reported that FOT may be useful in the detection of minimal changes inside of the respiratory system (49).

There was no significant difference between mean values of Rrs before and after which two pressure level applied to the airways (table 1). This response revealed poor contribution of recruitment effect and parallel ventilation of the PEEP below 5cmH2O. Following LS nasal stimulation, however significant differences observed the respect to RS and that of control groups.

Because of different penetration potency of the waves, medium amplitude- high frequency components are readily stopped soon after the large airways entrance, because of high energy dissipation rate (50). On the other hand, high amplitude- low frequency components are traveled long distance enough to meet the peripheral airways. So from the present findings, the overall increment in Zrs is an illustrative for our differentiation purpose, as postulated previously by Lutchen and Gillis(51). These authors introduced two distinct behaviors consist of homogeneous airway narrowing, with uniform increase in lung resistance (RL), and heterogeneous peripheral constriction, with a steep increase in RL over the lower band of the frequency range. In the present study, in the control group as in the intranasal stimulation group, mean Rrs tends to increase with frequency (lower band) which implies the result of heterogeneous peripheral airway narrowing in such a way that was significantly side-specific (50). Nevertheless the observed response in the control group is normally somewhat higher for low frequencies, the effect of stimulation explicitly augmented the frequency dependency both in RN and Xc. Steep decrement in Xc in the lower band is characteristically associated to decreased frangibility of peripheral airways in which capacitive restoration of energy is declined. Principally it might be due to diminution of bronchomotor tone or decrease in tethering tension around the airways (52).

Measurement of P-V loop provided a reliable viewpoint about inspiratory dynamics and development of intrinsic PEEP and over-distension. There was a statistically significant decrease in compliance in middle and final stages of the P-V curve following RS stimulation to that of the control group. Similar result was observed after LS stimulation but moderately right-shifted. Statistically significant successive difference also observed for initial, middle and final segments among two stimulation groups. Equally spaced UIP similar to LS group, but the absence of LIP in RS group is an indicator of development of intrinsic PEEP and partial collapse (Figure 4).

Quasi-static compliance which derived from the Xc in the control group, well satisfied a complex sigmoidal three parameter fitting criteria, with inverse exponential function for estimation at the overall behavior of the curves at significance limit of $p<0.001$. Complex formula could be written as follows.

$$f= a/(1+\exp(-(x-x0)/b)) \qquad (1)$$

Nasal stimulations showed non-satisfactory fitting with more complex formula, so it was performed only at simple exponential regression model (formula 1). As depicted in fig.4, even prediction with dedicated simple formula failed to ensure complete fitting on RS and LS stimulation compared to the calculated data. Rigorous underestimation is obviated upon fitting line over right-side data, whereas a fitting overestimation is prominent over the left-side. This fault is fitting, reasonably ensure the statistical difference between macroscopic characteristics and ones obtained following estimation from the frequency domain, which is in accordance with our hypothesis about the development of partitioning in lower frequencies and higher unit volumes.

It may be concluded here that unilateral nasal stimulations are likely associated with the macroscopic mechanical changes in the respiratory system. Hence that altered impedance spectra are the consequence of instantaneous and somewhat variable development of intrinsic PEEP and heterogeneity due to partitioning and differential changes in airway caliber.

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