

Determinants of airway hyperresponsiveness—Balance of tonic and phasic contractility of airway smooth muscles of lobular bronchioles

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ABSTRACT

Airway hyperresponsiveness (AHR) is a characteristic feature of asthma, and generally correlates with severity of asthma. Understanding the protection mechanism against excessive airway narrowing and how it breaks down is fundamental to solving the problem of asthma. In this paper we have proposed a stochastic modeling the airway smooth muscle bundle for reproducing AHR such as an increased sensitivity of the airways to an inhaled constrictor agonist, a steeper slope of the dose-response curve, and a greater maximal response to agonist. A large number N of contractile muscle cells was assumed to repeat themselves in between contraction and relaxation asynchronously. Dynamic equilibrium of statistic physics was applied to the system of ASM bundle. Thus, the relation of dose to response of a piece of ASM bundle was described by $\phi = \tanh(\beta H)$, where β was Boltzman factor and H represented energy of contraction induced by constrictor agents. Each of adjacent pair contractile cells was assumed to have Ising-type of antimagnetic interactions of preference energy J (for the condition of contraction-relaxation) between them. A motion equation for a piece of ASM bundle was described by $\dot{\phi} = N(H - zJ\phi)$, which explained existence of combined tonic and phasic contractions. Based on observations of Venegas *et al.* [4], airway responsiveness was assumed to be assessable by total volume of the ventilation defects (TVD) of 13NN PET-CT images. Interactions via propagation of Ca ion waves between ASM bundles would cause percolation probability by $p_\phi = (1 + \tanh(\beta H))^2 / 4$ along the tree, then the relation of dose (βH) to TVD was described by $TVD = p_\phi \left[1 - (1 - p_\phi)^3 / p_\phi^3 \right] - TVD_0$. TVD_0 represented the protection mechanism against

excessive airway narrowing, which was determined by the ratio of amplitudes between tonic and phasic contractions, thus the balance of amplitudes between tonic and phasic contractions of peripheral lobular smooth muscles would be the determinant of AHR.

Keywords: Airway Hyperresponsiveness; Stochastic Model of Airway Smooth Muscle; Ising-Type of Antimagnetic Interactions; Percolation Process; Phasic and Tonic Contractions

1. INTRODUCTION

The initial observation that bronchoconstriction occurs more rapidly in asthmatics when compared to non-asthmatics after exposure to a constrictor agonist was made in 1921 [1]. Tiffeneau and Beauvallet were the first to describe the use of acetylcholine inhalation tests to determine the degree of airway responsiveness in asthmatic patients in 1945 [2]. Now the methods for measuring airway hyperresponsiveness (AHR) have been standardized and are widely accepted [3].

AHR can be demonstrated in almost all patients with current symptomatic asthma. Using the standardized method, asthmatic subjects generally have a provocation concentration of methacholine or histamine causing a 20% fall in the forced expiratory volume in one second (FEV_{1.0}) PC₂₀ of <8 mg/mL. Most non-asthmatic subjects will have a PC₂₀ of >16 mg/mL. The severity of AHR generally correlates with the severity of asthma. Many different factors have been suggested to be involved in causing AHR.

AHR is complex in its mechanisms. One component of AHR is the acute transient and reversible AHR, which is closely associated with effects of acute airway inflammation. Although the link to airway inflammation is strong, the precise mechanism between airway inflammation and AHR are not entirely clear. The second, more

persistent component of AHR appears related to the chronicity of the disease and likely the chronic effects of airway inflammation leading to multitude of structural airway changes known as airway remodeling.

There is currently a major controversy among the molecular and cellular biologists and experts in the lung mechanics. The experts in lung mechanics feel that the disease will be not controlled until we understand the mechanics of excessive airway narrowing that characterizes asthma. From the point of view of a scientist trying to understand the reasons of abnormal airway narrowing in asthma, it is striking that airway smooth muscle (ASM) is capable of shortening to the extent that all airways within the lung could be completely closed. However, in the normal lungs the degree of narrowing cannot be provoked. Normal lungs are protected against excessive airway narrowing. A plateau develops on the bronchial dose response curve so that even an increase in dose of smooth muscle agonists of several orders of magnitude fails to produce an increased response. This protective mechanism is lost in asthma.

Venegas and his colleagues demonstrated the functional images of airflow obstruction as the ventilation defects (VD) during the test of AHR, which would reflect uneven constrictions of lobular bronchioles [4]. Brown and Mitzner suggested the plateau of the dose-response curve reflects uneven or limited aerosol delivery to the airways [5]. Another possibility is that mechanisms exist that act to limit the extent of muscle shortening in the health breathing lung, whereas these mechanisms become compromised in asthmatic lung. In this study, we have proposed another mechanism by a stochastic dynamic model of ASM to explain the protection mechanism against excessive airway narrowing in healthy lung and lost in asthmatic lung as well.

2. MODELING OF AIRWAY SMOOTH MUSCLE BUNDLE

2.1. Statistic Physics of Airway Smooth Muscle (ASM) Contractions

A large number of ASM cells compose the bronchial bundle (the ASM bundle) as a major contractile component of the bronchial tree. A piece of the ASM bundle with the degree of contraction ϕ was assumed to be composed of N cells which consist of m cells in contraction and n cells in relaxation ($N = n + m$), then two relations between the degree of contraction and the number of smooth muscles in contraction or relaxation were described as follows,

$$\frac{n}{N} = \frac{1 + \phi}{2} \tag{1}$$

$$\frac{m}{N} = \frac{1 - \phi}{2} \tag{2}$$

Each ASM cell was assumed to repeat itself asynchronously in between relaxation ($\sigma = -1$) and contraction ($\sigma = +1$). According to statistic physics of equilibrium [6], we would be able to find a ASM cell in the condition of σ by probability $P(\sigma)$ as follows,

$$P(\sigma) = \frac{e^{\sigma\beta H}}{e^{\beta H} + e^{-\beta H}} \tag{3}$$

where H was the field energy inducing contraction of ϕ , and β was Boltzman's thermal factor. Thus, for the ASM bundle with the degree of contraction ϕ and the field energy H , we obtained the equation as follows (**Figure 1**),

$$P(+1) = \frac{1 + \phi}{2} = \frac{e^{\beta H}}{e^{\beta H} + e^{-\beta H}}$$

or

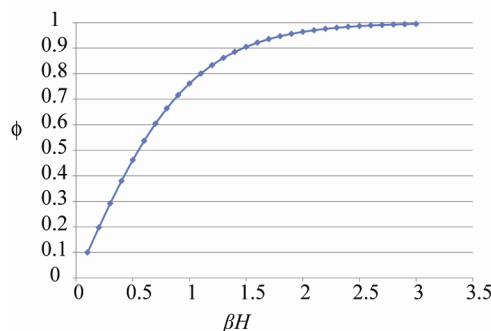
$$\phi = \frac{e^{\beta H} - e^{-\beta H}}{e^{\beta H} + e^{-\beta H}} = \tanh(\beta H) \tag{4}$$

2.2. Ising-Type of Antimagnetic Interactions between Adjacent Pair Cells

Adjacent pair cells were assumed to have an energetic preference J to be in the different conditions $(\sigma_i, \sigma_j) = (+1, -1)$ or $(-1, +1)$ (we called this Ising-type of antimagnetism interactions). The internal energy U of the piece of ASM bundle was able to be defined as follows,

$$U = \sum_{\langle i, j \rangle} J \sigma_i \sigma_j \tag{5}$$

where Σ of $\langle i, j \rangle$ indicated summation of adjacent pair cells across the whole piece of ASM bundle. If each ASM cell repeated itself asynchronously in between relaxation and contraction even in the piece of ASM bundle with the degree of contraction ϕ , we would be able to describe the internal energy $U(i, j)$ consisting of adjacent pair cells i and j in the probability of $P(\sigma_i, \sigma_j)$ as



The relation of dose (βH) to response (ϕ) is illustrated according to **Eq.4**. The dose of constrictive agonists is standardized by the parameter β , which is the thermodynamic factor changing the chemical agents to constrictive activities.

Figure 1. Standardized dose-response curve of ASM bundle.

follows,

$$P(+1,+1) = \left(\frac{1+\phi}{2}\right)^2, \quad P(-1,-1) = \left(\frac{1-\phi}{2}\right)^2$$

and

$$P(+1,-1) = P(-1,+1) = \frac{1+\phi}{2} \cdot \frac{1-\phi}{2}$$

Then,

$$U(i,j) = J\{P(+1,+1) + P(-1,-1)\} - J\{P(+1,-1) + P(-1,+1)\} = J\phi^2 \quad (6)$$

If each cell was surrounded by z adjacent cells, the internal energy of the piece of ASM bundle with the degree of contraction ϕ was able to be described by the following $U(\phi)$,

$$U(\phi) = \frac{NzJ\phi^2}{2} \quad (7)$$

Thus, the total energy E of this piece of ASM bundle with the degree of contraction ϕ under the contraction energy H was defined by the following equation,

$$E(\phi, H) = -N\phi H + \frac{1}{2} NzJ\phi^2 \quad (8)$$

2.3. Percolation Probability along Bronchial Tree

Inhaled constricting agonists such as methacholine or histamine would induce local tonic contractions of ASM, which would proceed to bronchoconstriction as a whole. Through asynchronous calcium ion waves among ASM cells local tonic contractions of ASM bundle would propagate to both peripheral and central parts of bundle along the binary branching tree in a stochastic manner (**Figure 2**). Thus, when the probability of propagation at the bifurcation (the site probability) was assumed to be equal to p in the whole branching tree, it was possible to describe the percolation probability of tonus along the bronchial tree $\theta(p)$ as follows [8],

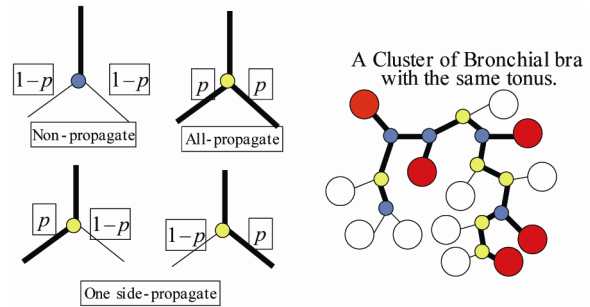
$$\theta(p) = p \left\{ 1 - \left(\frac{1-p}{p} \right)^3 \right\} \quad (9)$$

3. RESULTS

3.1. Combined Phasic and Tonic Contractions of the ASM Bundle

The differential equation for dynamics of ASM was obtained by use of **Eq.8**,

$$\ddot{\phi} = -\frac{\partial E}{\partial \phi} = NH - NzJ\phi \quad \text{or}$$



Propagation of tonus is regulated at the bifurcation of branching tree in a stochastic manner of the site probability of p .

A: Note that p and $(1-p)$ are permissive and non-permissive site probabilities at the bifurcation point, respectively. B: As a result, a cluster of connected terminals in red is obtained.

Figure 2. A stochastic process model of propagation of muscle tonus along a binary tree.

$$\frac{d^2}{dt^2} \left(\phi - \frac{H}{zJ} \right) = -NzJ \left(\phi - \frac{H}{zJ} \right) \quad (10a)$$

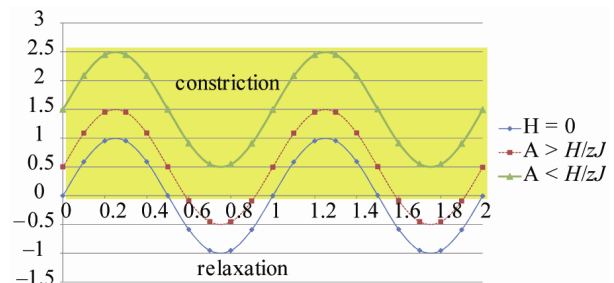
If the field potential H is zero, motions of ASM are oscillations (the phasic contractions with frequency of $\omega = \sqrt{NzJ}$). In general, dynamics of ASM bundles should be described by the combination of phasic and tonic contractions as follows,

$$\phi = A \sin(\omega t) + \frac{H}{zJ} \quad (10b)$$

Tonic contractions are cancelled when the amplitude of A is larger than H/zJ , and phasic ones are visible. Tonic contractions will be observed when A is less than H/zJ (**Figure 3**).

3.2. Constriction of Bronchial Tree as a Percolation of Tonic Contractions

If ASM bundles in adjacent pair branches appeared in the same contracted condition by the probability of $P(+1,+1)$, the site probability of p was assumed to be $P(+1,+1)$



Three types of phasic changes in the degree of contraction are demonstrated. Phasic relaxations can cancel tonic (persistent) contractions dependent on H during a cycle of changing when A is larger than H/zJ .

Figure 3. Balance of Combined Tonic and Phasic changes in the degree of contraction of ASM bundle at the Lobular bronchiole.

as follows (**Figure 4**),

$$p = P(+1, +1) = \left(\frac{1+\phi}{2}\right)^2 = p_\phi \quad (11)$$

Thus, based on **Eq.9** and **Eq.11** tonic contractions of the ASM bundle in the whole bronchial tree was described by the percolation probability $\theta(p_\phi)$ as follows,

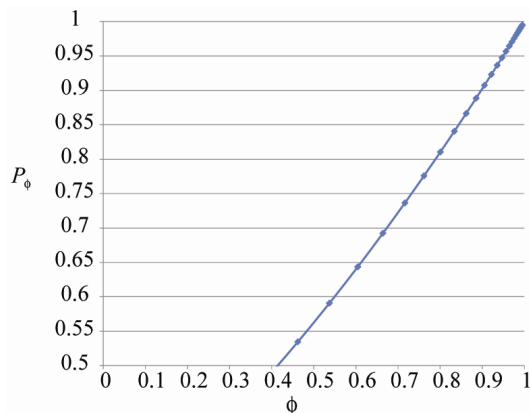
$$\theta(p_\phi) = p_\phi \left\{ 1 - \left(\frac{1-p_\phi}{p_\phi} \right)^3 \right\} \quad (12)$$

3.3. Determinants of Airway Hyperresponsiveness

Test of airway hyperresponsiveness (AHR) is designed for assessing the degree of tonic bronchoconstriction induced by inhaled methacholine or histamine. Bronchoconstriction is assessed by decrease in forced expiratory volume in one second (FEV_{1.0}). Recent functional images of asthma by Venegas and his colleagues showed the ventilation defects (VD) as peripheral bronchiolar obstructions [4]. Total volume of VD (TVD) was proportional to decrease in FEV₁₀, therefore the degree of bronchoconstriction was proportional to TVD. If TVD was caused by peripheral bronchiolar obstructions induced by inhaled constricting agonists, TVD would be expressed by the percolation probability $\theta(p_\phi)$ as follows,

$$\text{TVD} = \theta(p_\phi) = p_\phi \left\{ 1 - \left(\frac{1-p_\phi}{p_\phi} \right)^3 \right\} \quad (13)$$

The site probability p_ϕ was described by the equation from **Eq.4** as follows,



Note that the site probability P_ϕ in the percolation process should be more than 0.5 if a cluster appears. Thus, the degree of contraction ϕ has to be more than about 0.4.

Figure 4. The relation of the degree of ASM contraction to the site probability.

$$p_\phi = \left(\frac{1 + \tanh(\beta H)}{2} \right)^2 \quad (14)$$

If the maximal degree of bronchoconstriction was measured by TVD_{\max} during the provocation test, the parameter $\text{TVD}_0 = 1 - \text{TVD}_{\max}$ indicated the protection mechanisms of excessive airway narrowing. Thus, the test of provocation was described by **Eqs.15a, b** and **c** as follows,

$$\phi = \tanh(\beta H) \quad (15a)$$

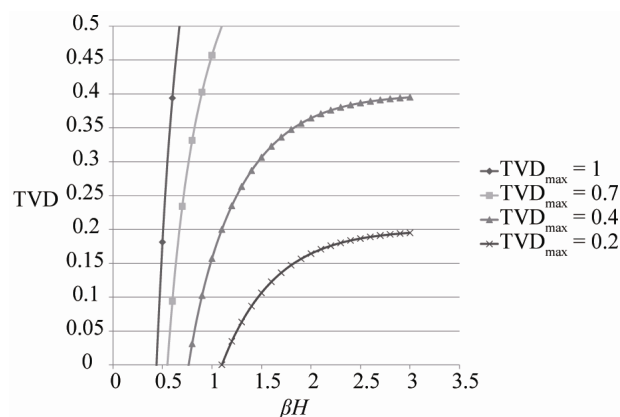
$$p_\phi = \left(\frac{1 + \tanh(\beta H)}{2} \right)^2 \quad (15b)$$

$$\text{TVD} = p_\phi \left\{ 1 - \left(\frac{1-p_\phi}{p_\phi} \right)^3 \right\} - \text{TVD}_0 \quad (15c)$$

We computed ϕ , p_ϕ , and TVD (at TVD_{\max} of 0.2, 0.4, 0.7, 1.0) in the domain of between $\beta H = 0$ and $\beta H = 3.0$ according to **Eqs.15a, b** and **c**, and demonstrated the relationships between βH and TVD in **Figure 5**. Simulated these curves reproduced clearly properties seen in the bronchial dose response curves of the change in FEV_{1.0} vs baseline induced by increasing dose of a bronchoconstrictor stimulus (methacholine) in patients with mild, moderate and severe asthmatics vs healthy individuals [9].

4. DISCUSSION

Previously reported studies on modeling dynamics of the airway smooth muscles (ASM) have been concentrated on changes in dynamic properties of the sub-cellular



AHR consists of an increased sensitivity of the airways to an inhaled constrictor agonist, a steeper slope of the dose-response curve, and a greater maximal response to agonist. TVD; total volume of the clusters of hypoventilated lobules detectable by Venegas' method of 13NN-PET-CT[4], TVD_{\max} ; maximal response to agonist of 1.0, 0.7, 0.4 and 0.2, βH ; the standardized activity of constrictor agonist.

Figure 5. Dose-response curves simulating Airway Hyperresponsiveness (AHR).

molecular elements such as the acto-myosin cross links or the cytoskeleton [10,12]. Many of the biophysical phenomena were observed from the strip of ASM tissue which is composed of a large number of cells. All of the biophysical properties of ASM, such as active force generation and shortening velocity, are based on data from experiments on various strips of ASM tissues. However, no discussion has thought of the multicellularity of the experimentally prepared materials until today. What we proposed in this study was the modeling based on the multicellularity of airway smooth muscle bundle, thus we were able to take account of using the concept of statistical physics on this ASM system (see **Eq.4**).

There is a well-known statistic physical model of Ising for describing ferromagnetism of materials [6,7]. Ising model in this study was assumed to have antiferromagnetic type of interactions among contractile cells (*i.e.*, the energy preference J was assumed in adjacent pair cells in different conditions such as contraction-relaxation). Then, the model for the ASM bundle we proposed revealed the existence of oscillations or phasic contractions. Recent studies on fetal lung explants have showed that airway peristalsis (AP) during morphogenesis, which appears in the first trimester of pregnancy and then increases in frequency towards birth (~ 1.0 cycle/min), is the spontaneous phasic propagation of waves of ASM contractility [12-15]. The existence of AP in fetal lungs would support the assumption of Ising-type of antiferromagnetic interactions among muscle cells.

In the healthy intact dog, ASM possesses sufficient force generating capacity to close all airways. But healthy animals or humans are challenged with inhaled contractile agonists in concentrations thought to be sufficient to activate the muscle maximally, resulting airway narrowing limited in extent. Brown and Mitzner [5] have suggested that the plateau of the dose-response curves reflects uneven or limited aerosol delivery to the airways. Venegas and colleagues [4] also demonstrated appearing of clusters of poorly ventilated pulmonary lobules (the ventilation defects, VD) in asthmatics at the induced airflow obstruction. The existence of VD suggested uneven tonic constriction of lobular bronchioles induced during airflow obstruction. However, we took another possibility of understanding the uneven bronchiolar constrictions. Based on the recent studies of calcium ion waves among muscle cells, we applied the stochastic process of transmission of ion waves along ASM cells to explain uneven constrictions of peripheral bronchioles. As the bronchial tree is a binary branching tree, the percolation probability $\theta(p)$ in **Eq.9** is obtained logically and was used for explaining the unevenness of bronchiolar constrictions. Bronchial thermoplasty represents an exciting new therapy for refractory asthma [16]. However, the mechanism for its effects is unclear. From our study, we propose the

hypothesis that bronchial thermoplasty achieves its distal airway effects by change in the percolation probability. But, now we have no data about the site probability p . Then, in this study we proposed to take the probability of adjacent branches in same tonic constrictions of **Eq.11** as p .

As described above, healthy animals or humans are challenged with inhaled contractile agonists in concentrations thought to be sufficient to activate the muscle maximally, resulting airway narrowing limited in extent. This is called the protection mechanism against excessive airway narrowing. Professor Peter Macklem [17] said "Understanding the protective mechanism and how it breaks down is, from the point of view of the physiologist, absolutely fundamental to solving the problem of asthma." Many extramuscular factors have been proposed such as the cartilage of the large bronchi, the elasticity of the airway wall, elastic tethering forces conferred by contractile cells in the lung parenchyma [18,19], mechanical coupling of airway to the parenchyma by the peribronchial adventitia, and buckling of the airway epithelium and submucosa [20-22], but no one can explain sufficiently the protection mechanism against the excessive airway narrowing. According to **Eq.10b**, phasic relaxations of ASM at the lobular bronchioles are able to cancel tonic constrictions induced by a field energy of H when the amplitude A is larger than H/zJ as shown in **Figure 3**, which would act as disappearance of VD. If increase in VD indicates the excessive airway narrowing, this mechanism of cancelling of VD would be the protection mechanism against the excessive airway narrowing. Thus, in this study we have proposed that the phasic change in tonus of ASM bundles over tonic contractions at the peripheral bronchioles is the major role of protection against excessive narrowing of bronchioles. The potential H would be composed of many extramuscular factors including airway inflammation. Although the determinants of amplitude A are unknown at all, it is necessary to describe in detail the determinants of parameter A for understanding the pathophysiology of chronic persistent asthma.

AHR has been known to consist of the sensitivity and the reactivity [9]. The sensitivity is determined by the initial dose of inhaled methacholine or histamine for induction of bronchoconstriction, and the reactivity is the relative increase in the degree of bronchoconstriction at inhalations of higher incremental dose. The relationships between TVD and βH in **Figure 4** showed correlated increases in the sensitivity and the reactivity among asthmatics, and the difference between asthmatics and normal lungs as well. These were explained by the difference in TVD_0 which was determined by the ratio of H to A . Therefore, the conclusion of this study is that AHR are dependent upon the protection mechanism against the

excessive airway narrowing, which is determined by balance of the phasic and tonic contraction-relaxation properties of ASM bundle under the level of potential energy consisting of many extramascular factors including airway inflammation.

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