REVIEW ARTICLE

BRONCHIAL HYPERREACTIVITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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INTRODUCTION

According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD), a scientific committee of National Heart, Lung, and Blood Institute, National Institutes of Health, USA, and the World Health Organization, the chronic obstructive pulmonary disease (COPD) is defined as "a disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases" [1]. Pathologically chronic bronchitis and emphysema are included in this category but airflow obstruction due to asthma, bronchiectasis and pulmonary infections like tuberculosis, is not included [2].

It is indeed rare to find a case of 'pure' COPD in the clinical setup. There are often patients in whom clinical picture overlaps with other respiratory disorders especially asthma. Consequently there are no rigid criteria to define and categorize COPD. One reason for this might be the existence of different clinical phenotypes of the disease. COPD presenting with bronchial hyperresponsiveness is possibly, one such phenotype.

Bronchial hyperresponsiveness (BHR) is defined as an "excessive bronchial narrowing and manifests itself as an exaggerated bronchoconstrictor response of the airways to various inhaled stimuli" [3]. The phenomenon of bronchial hyperresponsiveness first came to light in 1947 when it was documented that asthmatics show a greater bronchoconstrictor response when administered methacholine and histamine as compared to non asthmatics [4]. Later in 1957, threshold doses were used to calculate the response of the airways to chemical and allergic stimuli, so that the phenomenon could be properly tested [5].

Although considered traditionally, as indicative specifically of asthma, it has now been found that a substantial number of patients with other respiratory disorders especially COPD as well as asymptomatic individuals, exhibit airway hyperresponsiveness.

Measurement of BHR:

Standard procedures are being employed in hospitals worldwide in order to confirm the diagnosis of asthma as well as in respiratory research in order to investigate the phenomenon of BHR in diseases other than asthma.

Bronchial hyperreactivity can be measured by direct tests and indirect tests [3]. Direct tests use stimuli, which act directly on the airway smooth muscle (ASM) and causes bronchoconstriction. Examples include methacholine and histamine. Indirect tests use stimuli that act indirectly. These indirect stimuli first cause the release of inflammatory mediators, which in turn lead to airway smooth muscle (ASM) contraction directly, or stimulate neural pathways leading to bronchoconstriction for example; adenosine monophosphate (AMP), hypertonic saline, eucapnic hyperventilation, and exercise.

The two most commonly used methods for measuring BHR are Methacholine

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Challenge Test (MCT) and Exercise Challenge Test (ECT). Detailed guidelines have been provided by the American Thoracic Society for both these tests [6]. An in depth discussion of the various protocols is beyond the scope of this review. However, a brief description of MCT is given here. Two protocols are used to check reactivity to methacholine: 1) Two minute tidal volume breathing protocol and 2) Five breath dosimeter protocol.

1. ***Two minute tidal volume breathing protocol:***

   Methacholine is available in vials in powder form. It is mixed with sterile normal saline (0.9% sodium chloride) to produce ten different concentrations from 16 mg/ml to 0.031 mg/ml. After recording the baseline forced expiratory volume in the first second (FEV_1), the subject is nebulized with the lowest concentration of methacholine for two minutes using a specially designed nebulizer. Percentage fall in FEV_1 is noted. Increasing concentrations of methacholine are administered until FEV_1 falls by at least 20% and the concentration is noted.

2. ***Five breath dosimeter protocol:***

   In this method a different type of nebulizer is used. After recording the baseline FEV_1, the patient takes in five deep breaths of the lowest concentration of methacholine. FEV_1 is recorded 30 and 90 minutes after the manoeuvres. Higher concentrations of methacholine are used until fall in FEV_1 is 20% or more.

The results are reported as 'PC20' or the concentration of methacholine at which FEV_1 declined to 20% or less. It is interpreted as follows:

PC20 > 16 mg/ml = normal BHR

PC20 4-16 mg/ml = Borderline BHR

PC20 1.0-4.0 mg/ml= Mild BHR

PC20 < 1.0 mg/ml=Moderate to severe BHR

(Positive test)

All patients of asthma will have a positive test. As a matter of fact the Global Initiative for Asthma (GINA) has included BHR in the definition of asthma [7]. A small percentage of patients with other diseases also show bronchial hyperreactivity. However, it has been found that the phenomenon is also prevalent in the asymptomatic population. In the western countries, BHR to histamine or methacholine is found in 16–30% of children8 and in 10-16% of adults [9-11] with no respiratory symptoms. Risk factors for the development of BHR include poor baseline lung function, atopy, male sex in children and the female gender in adults, very young and very old age, smoking high salt intake and exposure to inorganic dust and products of pyrolysis [10, 12-15]. Trigger factors include air pollution [16] and respiratory infection [17].

PHYSIOLOGICAL MECHANISMS OF BHR

Since BHR has long been considered almost synonymous with asthma, most of the research regarding its pathophysiology has been conducted in asthmatic patients and animal models of asthma. The research done as yet on bronchial hyperresponsiveness in COPD has shown that the mechanism of BHR in COPD differs somewhat from BHR in asthma.

Airway Smooth Muscle (ASM) – The Central Player in BHR:

The airway smooth muscle (ASM) like all other muscles possesses actin and myosin filaments arranged in sarcomeres. Contraction occurs by the sliding filament mechanism. The distinguishing feature in smooth muscle contraction is the phosphorylation of myosin light chain before they can bind to actin. Only after the phosphorylation will cross bridge formation occur, and actin will slide on myosin. This phosphorylation is dependant

on the cytoplasmic enzyme, myosin light chain kinase (MLCK), which in turn is regulated via several mechanisms. The predominant mechanism is mediation via calcium. Calcium, which enters cells via voltage gated calcium channels and is also released from the sarcoplasmic reticulum (SER), combines with the regulatory protein calmodulin and the resulting complex activates myosin light chain kinase (MLCK). As MLCK phosphorylates myosin, actin myosin interaction takes place and the smooth muscle contracts. Bronchial hyperreactivity can be considered as an exaggerated process smooth muscle contraction in response to provocative stimuli.

But why does smooth muscle contract excessively? Again most of the attempts to answer this question have involved research on asthmatic patients and animals models of asthma. In asthma it has been found the excessive contraction maybe due to abnormality of smooth muscle itself or its environment. Airway smooth muscle abnormalities have been found to be hyperplasia, hypertrophy as well as increased levels of myosin light chain kinase [18, 19]. Factors in the environment of the smooth muscle causing increased activity of ASM and hence producing the hyperresponsiveness include inflammation and effect of extracellular matrix (ECM) proteins.

It has been shown that ECM proteins increase airway smooth muscle proliferation in vitro [20]. It has also been shown that the ECM proteins might help prevent apoptosis of the ASM [21]. However it is not known if this translates into increased smooth muscle mass in vivo and excessive bronchoconstriction leading to BHR.

Inflammation Stimulates Excessive Smooth Muscle Contraction Producing BHR:

Venkayya et al. conducted an experiment in which they administered a media containing activated $CD4^+$ T cells to the airways of naive mice. Bronchial hyperreactivity was produced and it was found that IL-4 and IL-13 (the cytokines produced by helper T type 2 cells) were important mediators of this response. Thus it was concluded that exaggerated helper T cell type 2 (Th2) responses might produce BHR generally [22]. In asthma, failure of Th1 cells to produce sufficient IFN-gamma along with excessive Th2 response is an essential prerequisite for bronchial hyperresponsiveness. Exaggerated Th2 response alone only produces atopy, without airway obstruction [23]. Increased amount of pro-inflammatory cytokines IL-5 and IL-8 in induced sputum from asymptomatic subject with BHR further proves that inflammation is an important cause of BHR [24]. Another cytokine, which might be involved in the development of airway hyperresponsiveness, is IL-8. Its administration has been shown to produce BHR in animal models [25].

Leukotrienes are also important mediators of BHR in animal models of allergic asthma. The cysteinyl-leukotrienes (Cys-LTC4, -LTD4, and -LTE4) have been shown to be potent bronchoconstrictors by acting directly on the smooth muscle receptor [26]. LTB4 on the other hand has no direct effect on the ASM. It is thought to produce BHR indirectly by promoting chemotaxis of inflammatory cells, maintaining the inflammatory response and increasing vascular permeability [27,28].

The Mechanism of BHR in COPD:

The mechanism of BHR in COPD has similarities with BHR in asthma. Some studies have reported an increase in the airway smooth muscle mass in COPD18. This increased smooth muscle mass might translate into increased force of contraction and airway hyperresponsiveness. Inflammation plays a primary role in

stimulating the excessive bronchoconstrictor response in COPD. As mentioned above IL-4 has been found to be an important mediator of asthmatic BHR [22]. It might also be involved in BHR of COPD, as the decline in FEV_1 (Forced expiratory volume in the first second) in COPD is associated with IL4 gene polymorphism [29]. Additionally it is also known that excess IL-13 (another cytokine involved in asthmatic BHR) favours proteolysis, inducing emphysema [30]. Thus it too might have a role to play in BHR of COPD. However helper T cells type 1 defect with associated insufficient IFN-gamma production, shown to be essential for BHR in asthma [23], might not have that important a role in BHR of COPD. As a matter of fact excessive IFN-gamma (produced by Th1 cells) has been shown to shift the protease-antiprotease balance to favour proteolysis and hence induce emphysema. It also inhibits the secretion of leukocyte protease inhibitor, further inducing emphysema [31]. Therefore a COPD patient is likely to have excessive IFN-γ even with BHR. Thus the exact role of IFN-γ in BHR associated with COPD is still not clear.

Parasympathetic / Cholinergic Dysfunction:

Acetylcholine (Ach) is released in the airways from parasympathetic nerve endings and non-neuronal origins [32]. When acetylcholine attaches to muscurinic receptors on the airway smooth muscle, the smooth muscle contracts. Thus it is logical to hypothesize that exaggeration of this physiological phenomenon might have a role in BHR. The clinical observation that anticholinergic drugs provide an effective bronchodilator therapy and the fact that they have also been shown to decrease airway hyperresponsiveness in COPD, reinforce this hypothesis [33]. Muscurinic receptors M2 and M3 are present on the cell membranes of airway smooth muscles (ASMs). M3 receptors are more important in the development of BHR as it has been observed that knocking out M3 and not M2 receptors protects mice from excessive bronchoconstriction in response to methacholine administration and vagal stimulation [34]. However cholinergic dysfunction might not be confined to the receptor level. Intracellular signalling pathways that eventually lead to actin myosin cross-bridge cycling might be abnormal.

Intracellular signalling involves calcium-dependant and calcium-independent pathways [35]. The main calcium dependant pathways are the phospholipase C (PLC)/ Inositol triphosphate (IP3) pathway and the CD38/cyclice adenosine dinucleotide phosphate ribose (cADPR) pathway. The attachment of Ach to its G-protein coupled receptor induces the activation of phospholipase C (PLC) leading to the formation of Inositol triphosphate (IP3). IP3 causes the release of calcium ions from sarcoplasmic reticulum (SER). At the same time that PLC is stimulated, another protein CD38 also becomes activated. It in turn activates cyclic ADP ribose, which too causes calcium release from SER.

The calcium independent pathways act by inactivating the enzyme myosin light chain phosphatase (MLC-P). MLC-P normally limits contraction by dephosphorylating the myosin head, detaching it from actin and cause muscle relaxation. The primary calcium independent pathway is the RhoA/ Rho kinase pathway. When Ach binds it's G protein coupled muscurinic receptor, intracellular protein RhoA is activated. RhoA activates the enzyme Rho kinase, which in turn inhibits myosin light chain phosphatase. The myosin light chain thus remains phosphorylated and continues to bind actin leading to sustained contraction [35]. It is possible that enhancement of anyone or all of these pathways may lead to BHR [36] (fig. 1).

It is well known that cholinergic activity is increased in chronic obstructive pulmonary disease. Airway inflammation, which is an integral component of the pathology of

COPD, seems to be partly responsible for this phenomenon. Inflammatory mediators like tachykinins, thromboxane A2 and prostaglandins can increase the release of acetylcholine from parasympathetic nerve endings [37]. Additionally mediators of inflammation including IL-1β [38], IL-13 [39], TNF-α [40] and IFN-γ [41] can augment the CD-38/cADPR pathway. All these mediators are increased in the airways in COPD and anyone or all of them might increase the cholinergic activity. Likewise it has been recently discovered that RhoA/RhoK kinase pathway itself, is enhanced in animals exhibiting BHR due to chronic exposure to cigarette smoke [42]. Thus enhanced RhoA / RhoK might be an important mechanism for the development of bronchial hyperresponsiveness in smokers with COPD.

Role of Tachykinins:

Tachykinins, like substance P and neurokinins, can cause airway smooth muscle contraction leading to BHR. The C-fibre afferents of the bronchial mucosa are stimulated by various chemical stimuli as well as cold air. When stimulated, they cause bronchoconstriction by increased parasympathetic activity as well as the release of tachykinins. Increased activity of tachykinins could be a contributing factor in the development of BHR. As a matter of fact it has been recently discovered that tachykinins and their receptors are involved in BHR due to cigarette smoke exposure in animal models [43]. Thus increased tachykinin activity is

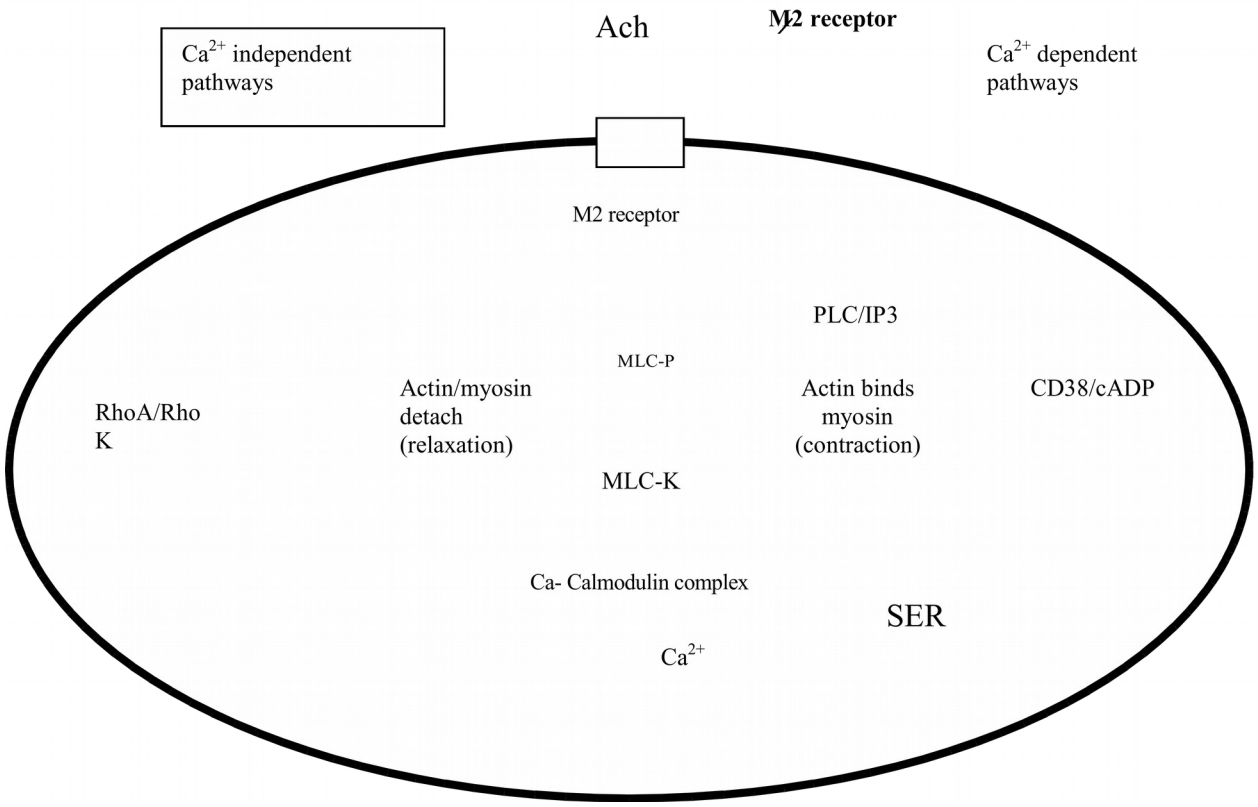

Figure: The airway smooth muscle has the M2 muscurinic receptor on its membrane.

Explanation of Figure: When attached with its ligand, acetylcholine (Ach), two types of intracellular signalling pathways are triggered. RhoA/Rho kinase (RhoA/RhoK) pathway inhibits myosin light chain phosphatase (MLC-P), where as the phosholipase C / inositol triphosphate (PLC/IP3) and CD38/cyclic ADP ribose (CD38/cADPR) pathways activate the rynodine receptors on the smooth muscle sarcoplasmic reticulum (SER) increasing the intracellular calcium (Ca2+) levels. The calcium then activates myosin light chain kinase (MLC-K) ultimately leading to contraction.

another attractive hypothesis to explain the development of BHR of smoking induced COPD.

Why to bother about BHR in COPD?

A recent study has proven that BHR is a definite risk factor for COPD. Around 500 patients of asymptomatic BHR were followed up for 11 years and it was found that smokers, current and former had a significantly high decline of FEV_1, bronchial hyperresponsiveness and the development of asthma and COPD [44]. Thus asymptomatic smokers exhibiting BHR are more likely to develop COPD than others. In addition to being a major risk factor for the development of this fatal disease, it is also an indicator of poor prognosis in COPD patients. Patients of COPD exhibiting BHR experience a faster decline in their lung functions. A study has shown that when smokers with BHR quitted smoking, their lung functions improved more than smokers who did not demonstrate BHR in first place [45]. Moreover, the presence of BHR in COPD is associated with increased mortality especially in smokers. There seems to be a relationship between the degree of BHR and the risk or death [46].

Hence information regarding the BHR status of a patient can be extremely useful for identifying high-risk groups in the general population and also for the management of COPD. COPD presenting with BHR might as well represent a distinct phenotype of the disease.

CONCLUSION

Patients of chronic obstructive airway disease, who exhibit BHR, represent a distinct subgroup of COPD patients. The airway hyperresponsiveness is primarily the result of excessive airway smooth muscle contraction due to complex molecular mechanism. It is a risk factor for COPD and associated with increased morbidity and mortality. As the pathophysiolgical mechanisms of BHR in COPD are uncovered, a better understanding can be gained of this particular phenotype of COPD and better therapies formulated.

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