Mechanisms of Bronchial Hyperreactivity in Asthma and Chronic Obstructive Pulmonary Disease

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Bronchial hyperreactivity has long been recognized as a hallmark of chronic asthma. Less is known about the prevalence and mechanisms of hyperreactivity in chronic obstructive pulmonary disease (COPD). Currently, it is unclear whether the role and mechanisms of hyperreactivity are similar in patients with asthma and COPD or whether the underlying pathophysiologic abnormalities are different for both diseases. The aim of this review is to present an overview of current knowledge of the mechanisms of bronchial hyperreactivity in asthma and COPD.

Keywords: asthma; chronic obstructive pulmonary disease; airway hyperresponsiveness; inflammation; human airway smooth muscle

Bronchial hyperresponsiveness (BHR) is defined as excessive bronchial narrowing and manifests itself as an exaggerated bronchoconstrictor response of the airways to various inhaled stimuli (1). It is considered to be a hallmark of inflammation in asthma, is related to the severity of the disease, and is increasingly being recognized as a clinical endpoint for therapeutic intervention. Less is known about the mechanisms and/or prevalence of BHR in patients with chronic obstructive pulmonary disease (COPD).

In the early 1960s, the “Dutch Hypothesis” stated that asthma and COPD should be considered as different expressions of one disease entity, in which both endogenous (host) and exogenous (environmental) factors play roles in the pathogenesis. Predisposition to develop BHR and allergy were considered to be important denominators of disease susceptibility. According to this hypothesis, the phenotype of the patient is the result of a combination of genetic and environmental factors, modulated by age and sex (2).

Since the 1960s and the formulation of the Dutch Hypothesis, much knowledge has been gained on the etiology and natural history of asthma and COPD. After the introduction of inhaled corticosteroids (ICS) for the treatment of asthma, it became generally accepted that airways inflammation is associated with BHR (3) and might even be considered an epiphénomènon of airways inflammation. Research during the 1990s in the area of cellular and molecular biology suggested that abnormalities of airway smooth muscle (ASM) cells rather than inflammation are the distinct cellular bases for BHR (4).

Currently, BHR is still considered as an important feature of asthma and COPD. However, evidence is accumulating that there are differences in BHR between these two airway diseases. This article presents an overview on current knowledge of the mechanisms of BHR in asthma and COPD.

The dose–response curve to inhaled provoking stimuli is characterized by different features, including the position (sensitivity), the slope (reactivity), and the maximal response plateau (1). In subjects with asthma as well as in COPD patients, the position of the dose–response curve is shifted to the left as compared with healthy subjects, indicating an increased sensitivity to the inhaled stimulus (5). In subjects with asthma, the slope of the dose–response curve is usually steeper as compared with that in healthy subjects (6), with COPD patients having a slope in between that found in asthma and healthy subjects (5). After inhalation of increasing doses of provoking stimuli, a plateau in the fall in FEV₁ can be observed in healthy subjects (6) and in most patients with COPD (7), whereas this is frequently absent in subjects with asthma (6). There are indications that factors contributing to maximal airway narrowing differ from those leading to increased sensitivity, at least in patients with asthma: Although the intensity of inflammation is associated with airway sensitivity, airway wall thickness seems associated with airway reactivity (8). Although it has been argued that both components should be assessed separately because they provide complementary information (9), epidemiologic studies mainly report one measure to indicate BHR: the provocative dose or concentration causing a 20% fall in FEV₁ (1).

Stimuli to Measure BHR in Asthma and COPD

BHR can be measured by using various bronchoconstrictors. These can be classified into two categories: stimuli such as histamine and methacholine that act directly on smooth muscle and those that act indirectly by stimulating the release of inflammatory mediators and/or by stimulating neural pathways (1). The latter such agents include AMP, hypertonic saline, eucapnic hyperventilation, and exercise (10). Because challenge tests with histamine and methacholine are well standardized, these are most frequently used in clinical settings and epidemiologic studies. Despite different methods, frequently accepted threshold levels for “normal” BHR are a provocative concentration causing a 20% fall in FEV₁ histamine or methacholine of 8 mg/ml or more or a provocative dose causing a 20% fall in FEV₁ histamine or methacholine of 7.8 μmol of more (1).

Differentiation Between Asthma and COPD Based on BHR

Although histamine and methacholine are the most frequently used nonspecific agents for BHR testing, neither agent seems a sensitive tool to discriminate between asthma and COPD. Therefore, interest has also focused on other challenge tests in research settings (11). In general, subjects with COPD do not respond to eucapnic hyperventilation (12), whereas subjects with asthma do (13), suggesting discriminative properties for this test between asthma and COPD. In children, BHR to AMP can be used to differentiate between the diagnoses of asthma, pediatric COPD such as cystic fibrosis, bronchiolitis obliterans, primary ciliary dyskinesia, and bronchiectasis, and healthy subjects (14).
TABLE 1. PREVALENCE AND RISK FACTORS OF/ FOR BRONCHIAL HYPERRESPONSIVENESS IN THE GENERAL POPULATION

<table>
<thead>
<tr>
<th>Reference</th>
<th>Definition BHR</th>
<th>Population</th>
<th>Prevalence BHR</th>
<th>Risk Factors or Associated with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>PD_{20} \leq 7.8- \mu\text{mol histamine}</td>
<td>Random sample of school children (n = 2,053)</td>
<td>15.9%</td>
<td>Wheeze, asthma</td>
</tr>
<tr>
<td>22</td>
<td>PD_{20} \leq 12- \mu\text{mol methacholine}</td>
<td>Random sample of school children (n = 388)</td>
<td>25–33%</td>
<td>Sex, positive skin test to HDM EIB, exposure to gas cooking</td>
</tr>
<tr>
<td>25</td>
<td>PD_{20} \leq 23-mL 4.5% hypertonic saline</td>
<td>Random sample of school children (n = 613)</td>
<td>14%</td>
<td>Atopic dermatitis, asthma, wheeze, sex</td>
</tr>
<tr>
<td>36</td>
<td>PD_{20} \leq 3.9- \mu\text{mol histamine}</td>
<td>Random sample of school children (n = 180)</td>
<td>16.1%</td>
<td>Serum IgE, skin test positivity</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>PD_{20} \leq 8- \mu\text{mol histamine}</td>
<td>Random population sample (n = 511)</td>
<td>14%</td>
<td>Skin sensitivity, smoking status</td>
</tr>
<tr>
<td>19</td>
<td>PD_{20} \leq 12.25- \mu\text{mol methacholine}</td>
<td>Random population sample (n = 2,415)</td>
<td>13%</td>
<td>Atopy, airway caliber, sex, age</td>
</tr>
<tr>
<td>20</td>
<td>PD_{20} \leq 3.9- \mu\text{mol histamine}</td>
<td>Random population sample (n = 922)</td>
<td>10.5%</td>
<td>Atopy, smoking status, respiratory symptoms, abnormal lung function</td>
</tr>
<tr>
<td>23</td>
<td>PC_{20} \leq 8-mg/mL histamine</td>
<td>Random population sample (n = 2,684)</td>
<td>18%</td>
<td>Respiratory symptoms, asthma attack</td>
</tr>
<tr>
<td>26</td>
<td>Fall in FEV₁/FVC &gt; 9%, 4 min of cold air</td>
<td>Random population sample (n = 134 adults, n = 213 children)</td>
<td>16%</td>
<td>Age, asthma</td>
</tr>
<tr>
<td>34</td>
<td>PC_{20} \leq 8-mg/mL histamine</td>
<td>Random population sample (n = 620)</td>
<td>39.4%</td>
<td>Eosinophilia, skin test positivity</td>
</tr>
<tr>
<td>42</td>
<td>PD_{20} \leq 4-mg/mL methacholine</td>
<td>General population sample (n = 799)</td>
<td>33.7% in females</td>
<td>Asthma, atopy, sex</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>11.9% in males</td>
<td></td>
</tr>
</tbody>
</table>

Definition of abbreviations: BHR = bronchial hyperresponsiveness; EIB = exercise-induced bronchoconstriction; HDM = house dust mite; PC = provocative concentration; PD = provocative dose.
1 g histamine = 3.26 mmol; 1 g methacholine bromide = 4.17 mmol.

Circumstantial evidence indicates that this is not the case in adults, as the degrees of BHR to both AMP and methacholine are comparable between nonsmoking subjects with asthma and smoking subjects COPD, although ex-smokers with COPD appear to be less sensitive to AMP inhalation (15). More recently, it has been demonstrated that subjects with asthma are more likely to exhibit hyperreactivity to methacholine than to AMP, which would render BHR testing with methacholine a more sensitive tool in the screening of populations for asthma than AMP (16).

EPIDEMIOLOGY OF BRONCHIAL HYPERREACTIVITY IN ASTHMA AND COPD

In the general population, BHR to histamine or methacholine is found in 16–30% of children (17) and in 10–16% of adults (Table 1 and 2) (18–24). Similar prevalence rates of BHR in the general population have been reported with other nonspecific stimuli: 14% of subjects have BHR to hypertonic saline (25), and 16% of subjects respond positively to cold air inhalation (26).

BHR is strongly associated with respiratory symptoms. Prevalence rates are therefore much higher in patient populations with asthma or COPD than in the general population. The definition of asthma states that the chronic inflammation of the airways, as seen in subjects with asthma, causes an associated increase in airway responsiveness (27). Indeed, in early studies, it has been reported that all patients with asthma are hyperreactive to histamine (28). Several epidemiologic studies in the mid-80s have estimated a much lower point prevalence of BHR in subjects with asthma, and prevalence rates of approximately 40–53% have repeatedly been established with different stimuli in these patients (17, 25, 26, 29). These differences in prevalence rates are most likely due to the populations studied: Cockcroft and

TABLE 2. PREVALENCE AND RISK FACTORS OF/ FOR BRONCHIAL HYPERRESPONSIVENESS IN SELECTED POPULATIONS

<table>
<thead>
<tr>
<th>Reference</th>
<th>Definition BHR</th>
<th>Population</th>
<th>Prevalence BHR</th>
<th>Risk Factors or Associated with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>PD_{20} \leq 25-mg/mL methacholine</td>
<td>Participants of the Normative Aging Study (n = 458)</td>
<td>29.9%</td>
<td>Smoking status, wheeze, FEV₁</td>
</tr>
<tr>
<td>33</td>
<td>PC_{20} \leq 16-mg/mL histamine</td>
<td>Healthy males (n = 227)</td>
<td>23.3%</td>
<td>Smoking, FEV₁</td>
</tr>
<tr>
<td>21</td>
<td>PC_{20} \leq 8-mg/mL methacholine</td>
<td>Male participants of occupational health surveys (n = 733)</td>
<td>11.4%</td>
<td>Atopy, smoking, accelerated rate of decline in FEV₁</td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>PD_{20} \leq 400- \mu\text{g methacholine}</td>
<td>Random sample of school children (n = 217)</td>
<td>47.1%</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>PC_{20} \leq 8-mg/mL histamine</td>
<td>Patients with asthma (n = 140)</td>
<td>100%</td>
<td>FEV₁</td>
</tr>
<tr>
<td>37</td>
<td>PC_{20} \leq 8-mg/mL methacholine</td>
<td>Patients with asthma (n = 214)</td>
<td>63.1%</td>
<td>IgE, eosinophilia, skin test reactivity</td>
</tr>
<tr>
<td>COPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>PD_{20} \leq 3.9- \mu\text{mol histamine}</td>
<td>Patients with COPD (n = 57)</td>
<td>46%</td>
<td>Airway caliber</td>
</tr>
<tr>
<td>31</td>
<td>PD_{20} \leq 5-mg/mL methacholine</td>
<td>Patients with mild COPD (n = 5,887)</td>
<td>25.4% in males</td>
<td>Sex, airway caliber</td>
</tr>
<tr>
<td>32</td>
<td>PD_{20} \leq 25-mg/mL methacholine</td>
<td>Patients with mild COPD (n = 5,666)</td>
<td>59% in males</td>
<td>Airway caliber, respiratory symptoms, sex</td>
</tr>
<tr>
<td>RADS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td>PC_{20} \leq 8-mg/mL methacholine</td>
<td>Firefighters from WTC (n = 102)</td>
<td>31%</td>
<td>Exposure to respiratory irritants</td>
</tr>
</tbody>
</table>

Definition of abbreviations: BHR = bronchial hyperresponsiveness; COPD = chronic obstructive pulmonary disease; PC = provocative concentration; PD = provocative dose or concentration; RADS = reactive airways dysfunction syndrome; WTC = World Trade Center.
1 g histamine = 3.26 mmol; 1 g methacholine bromide = 4.17 mmol.
colleagues studied patients with an established doctors diagnosis of (current) asthma (28), whereas the more recent epidemiologic studies related to a history of asthma or wheeze (17, 25, 26, 29).

Fewer studies have examined the prevalence of BHR in patients with COPD. Interestingly, the occurrence of BHR in COPD seems equal to or may even be higher than in asthma patients. In a limited number of subjects with mild COPD, a prevalence of BHR of 46% has been described (30). A similar prevalence rate, 25.4–47.8%, has been reported in subjects participating in the Lung Health Study, who also had mild COPD (31). This rate further increases when looking at mild BHR, the threshold concentration being defined as 25 mg/ml or less of methacholine: 59–63% of male participants and 85–87% of female participants exhibited mild BHR to methacholine (31, 32).

Smoking per se seems to influence the responsiveness of the airways because prevalence rates of 30–40% in current smokers and 18–25% in ex-smokers without airways obstruction have been described (24, 33). Although it has been argued that BHR is inherently determined by baseline FEV₁ (19), patients in the Lung Health Study had relatively good lung function, further underlining the high prevalence rates of BHR in COPD.

**RISK FACTORS FOR THE DEVELOPMENT OF BHR**

Large epidemiologic studies have identified several risk factors for the development of BHR (to histamine or methacholine). As such, asymptomatic BHR shows a complex relationship with several factors, including lung function, atopy, sex, age, and smoking (Tables 1 and 2).

The most important and independent risk factors for BHR are lung function and atopy. Lower FEV₁, a lower Tiffeneau ratio (FEV₁ as a percentage of FVC), and the presence of atopy are associated with an increased occurrence of BHR (18–22). The association between BHR and reduced airway caliber (caused by smooth muscle contraction, edema, or remodeling) can be explained by several features. Poiseuille’s Law states that airflow resistance is inversely related to the fourth power of the radius of an airway. Thus, the same degree of airway constriction will lead to a greater increase in airway resistance in smaller (obstructed) airways than in larger (unobstructed) airways. Furthermore, increased thickness of the airway wall, as can be observed in asthma (8) and COPD (34), leads to greater encroachment of the lumen for a given degree of airway constriction. Finally, the external load on ASM, including the relative stiffness of the bronchial cartilage and tethering effect of elastic recoil pressure, may oppose to a greater or lesser extent ASM contraction. Together, this may suggest that the presence of BHR in patients with airflow limitation is primarily an epiphenomenon of low baseline lung function and not due to inherently increased sensitivity of the ASM to inhaled stimuli.

The association between atopy and BHR seems more complex. Thus, it has been shown that atopy as a risk factor for BHR includes peripheral blood eosinophilia and skin prick test positivity, but not increased levels of IgE in serum (35). In contrast, others have shown that both skin prick test positivity as well as serum IgE is positively associated with BHR (36, 37). In further support for the relationship between BHR and atopy, it has been shown that BHR is frequently present in subjects without asthma with allergic rhinitis (38). Moreover, BHR worsens during the allergen season in subjects with asthma who are atopic to house dust mite (39), as well as in subjects with rhinitis who are atopic to pollen (38). In atopic subjects, the TH1/TH2 lymphocyte balance seems tipped in favor of overexpression of TH2 (40). The concomitant changes in cytokine release profiles, in combination with peripheral blood eosinophilia, may be involved in causing BHR to a variety of specific and nonspecific stimuli (41).

Several studies have shown a relationship between BHR and sex (19, 22, 31, 35, 42), although this association has not been confirmed by other investigators (20). In children without asthma, BHR is approximately twice as frequent in boys as in girls (22). On the other hand, the occurrence of BHR is more frequent among female than among male adults (19, 42). This switch in prevalence among males and females seems to occur during and/or after puberty (22). However, the major determinants of BHR appear to be the same before and after onset of puberty, which would argue against a role of hormonal changes on BHR during this period in life (22). Although it is still debated why the occurrence of BHR is different between men and women, it has been suggested that women are more susceptible to tobacco smoke and therefore more prone to the development of BHR (42). Furthermore, women have smaller airways than males, which renders them more likely to have measurable BHR compared with males, even for the same degree of airways obstruction (31).

The relationship between BHR and age remains unclear: It has been demonstrated that BHR is either not influenced by age (20) or that those of older age have a lower risk for BHR after correction for lung function (19) or that subjects of older age are more likely to have increased BHR (43).

Although smoking has been significantly associated with BHR (18–20), cigarette smoking appears less important than airway caliber and atopic status after correction for other risk factors (19). The current concept is that smoking leads to an increase in inflammatory cell numbers in the airways (44), which may in turn induce BHR.

Recently, other risk factors for the development of BHR have been identified. BHR can be triggered by inhalation of inorganic dust, products of pyrolysis, and other respirable materials, as has been shown in firefighters from the World Trade Center site (45). Approximately 30% of these firefighters developed BHR (45), which appeared to be associated with exposure intensity, independent of smoking status and airflow obstruction (46). In general, it seems that inhalant irritants can transiently or permanently induce BHR (47).

**What Does This Mean for Patients with Asthma and COPD?**

In the general population, lower lung function, being atopic, female sex (among adults), and smoking are more frequently associated with the presence of BHR. It is unknown what the exact interplay between each of these risk factors is in relationship to asthma and COPD. Interestingly, the same risk factors that have been identified for the development of BHR have been implicated as important factors in the etiology of these two airways diseases. In COPD, for example, smoking has been identified as the most important risk factor for the development and progression of COPD (48), whereas the presence of atopy (49), BHR (50, 51) and female sex (52) have been associated with a poorer prognosis, illustrating the complexity of these risk factors.

**BHR AS RISK FACTOR FOR ASTHMA AND COPD**

It is now well established that BHR is an important feature of obstructive lung diseases, including allergic asthma and COPD (27, 53). In particular, BHR is considered as a risk factor for both the development (23, 54) and progression (33, 55, 56) of asthma and COPD. Follow-up studies of patients with asthma or COPD have shown that BHR follows a different pattern in these disorders. BHR is associated with the annual rate of decline in FEV₁ both in patients with asthma (55) and COPD (50, 57).
Figure 1. Methacholine reactivity is associated with the mean change in postbronchodilator FEV₁ during 5 years of follow-up among smoking subjects with minimal, mild, and moderate airway obstruction in the Lung Health Study. Smokers with the most reactive airways have the greatest annual decline in FEV₁. This association appears to be the strongest among subjects with the most airways obstruction at entry of the study (moderate). Dashed line = subjects with minimal or borderline airways obstruction; dotted line = subjects with moderate airways obstruction. (Adapted by permission from Tashkin and colleagues [50].)

(Adapted by permission from Tashkin and colleagues [50].)

**TRIGGER FACTORS FOR BHR**

Although several risk factors for the development of BHR have been identified, other exposures have been implicated in temporary changes in airway reactivity. BHR may be variable over time because of several reasons. Experimental exposure to allergen of subjects with asthma is associated with an increase in BHR the following day (1). Seasonal variations in the house dust mite allergen load and air pollution are paralleled by fluctuations in BHR, with higher house dust mite and air pollution levels being associated with increased responsiveness (39, 59). Furthermore, in subjects with asthma, a worsening in indices of BHR has been observed after a respiratory infection (60). Again, in subjects with COPD, less is known about the factors associated with aggravation of BHR than in subjects with asthma. Specifically, the role of microbial colonization of the airways in COPD in relationship to BHR has so far not been addressed.

**AIRWAY INFLAMMATION IN RELATIONSHIP TO BHR IN ASTHMA AND COPD**

BHR has often been linked to airways inflammation, even in subjects who are asymptomatic (61). In patients with asthma and COPD, airways inflammation is present in the large and small airways (27, 53) but usually has distinctly different characteristics between the two patient populations (62). That is, the inflammatory profile in asthma is characterized by eosinophilia in airway wall mucosa (63, 64) and sputum (65), with additional neutrophilia in patients with more severe asthma (66, 67), during exacerbations (68), and in fatal attacks of asthma (69). In contrast, in COPD patients, airway inflammation is dominated by neutrophilia in sputum (70), with additional eosinophilia in the mucosa of bronchial biopsies and in sputum of patients with more severe COPD (71) and during exacerbations (72).

**Is Airway Inflammation Associated with Responsiveness of the Airways?**

In subjects with asthma, the level of BHR to histamine or methacholine is related to the numbers of mast cells, CD8⁺ T cells, and eosinophils in the lamina propria (73), as well as with the number of eosinophils in induced sputum (74, 75). Thus, more severe BHR is associated with a more intense inflammatory profile in subjects with asthma. Because inhalation of AMP induces bronchoconstriction through cellular pathways (11), it can be expected that the relationship between airways inflammation and AMP responsiveness is a closer one than that with BHR to histamine or methacholine. Indeed, in a large study, including 120 patients with asthma, it has been shown that BHR to AMP and methacholine are both associated with sputum eosinophil numbers, but the correlation coefficient was higher for BHR to AMP than for methacholine (76). Moreover, after adjustment for other variables, it appeared that BHR to AMP is predicted by sputum eosinophilia, with baseline FEV₁ being another independent predictor. BHR to methacholine was predicted only by FEV₁ in subjects with asthma (76). However, still “only” 16% of the total variation in BHR to methacholine is explained by sputum eosinophil numbers (75), again illustrating that no single factor causes BHR in asthma.

Only a few studies have investigated the direct relationship between airways inflammation and BHR in COPD. One recent report in smoking subjects with COPD shows that indices of BHR are not related to measures of impairment of the lung parenchyma structure, as determined by pressure–volume curves and carbon monoxide diffusion (77). This may suggest that BHR in smoking COPD patients is determined by airway pathology rather than parenchymal impairment (77). Another study has demonstrated that the presence of BHR to AMP in subjects with COPD is associated with increased numbers of CD8⁺ T cells in the lamina propria in the large airways and with higher eosinophil counts in induced sputum samples (78). Furthermore, in subjects with centrilobular emphysema, the number of T lymphocytes in the airway wall is associated with BHR (79). Clearly, the issue of airways inflammation in association with the extent of BHR deserves further investigation in COPD patients.

**CELLULAR MECHANISMS UNDERLYING BHR**

**How Can These Associations between Airways Inflammation and BHR Be Explained?**

Extensive studies of human bronchial rings *ex vivo* and animal studies *in vivo* have explored the cellular mechanisms underlying BHR. Early studies combining the measurement of BHR of the airways *in vivo* with that of bronchial smooth muscle strips from surgical specimens from the same patients *in vitro* have shown that indices of BHR *in vivo* and *in vitro* are correlated in neither patients with asthma (80) nor patients with COPD (81). This suggested that BHR is not due to pathophysologic changes in the ASM cells, but, rather, that some aspect of the ASM microenvironment *in vivo* is necessary for induction and mainte-
nance of BHR (80, 81). Later investigations have looked at possible candidates for this micromilieu and have examined the role of IgE and mast cell tryptase. Bronchial rings from subjects with high circulating IgE appear to be more responsive to contractile stimuli than rings from subjects with low IgE (82). Interestingly, the responsiveness of bronchial rings from subjects with low IgE increases after incubation with IgE-rich serum (82). In addition, bronchial rings from current smokers are more responsive to histamine-induced contraction than similar preparations from nonsmokers and ex-smokers, and responsiveness can be increased further in both groups by incubation with IgE-rich serum (83). This suggests that smoking is involved in the development of BHR independently of IgE, which is in agreement with epidemiologic studies. The contractile response of bronchial rings isolated from sensitized humans can be potentiated further by the addition of human mast cell tryptase (84). Together, these studies show that isolated ASM can be modified by exogenous factors to exhibit BHR, suggesting a specific role for smooth muscle cells in this phenomenon.

In addition to IgE, it is recognized that CD4+ T lymphocytes and their cytokine products, including interleukin (IL)-4, IL-5, and IL-13, are important in the pathogenesis of asthma and the induction and/or maintenance of BHR (85). Cytokines from activated CD4+ T lymphocytes, differentiated toward a Th2 profile, have been shown to be implicated in the development of BHR in a mouse model of asthma (86). In particular, IL-4 and IL-13 seem to regulate this response (86). After inhalation of allergen by sensitized mice, CD4+ cells infiltrate the airways, followed by accumulation and degranulation of eosinophils and BHR (87), which appears to depend totally on IL-5 (87, 88). Mast cells have a bystander role in the recruitment of eosinophils on allergen inhalation but do not appear to be involved in BHR in mice (89) (Figure 2).

In animal models of asthma, it seems that normal expression of Th1 cells can reduce allergen-induced BHR, which is in part regulated through IFN-γ production (90). Interestingly, in adult subjects, it has recently been demonstrated that an impaired response of Th1 cells to produce IFN-γ, in addition to overexpression of Th2 profile cytokines, is associated with the persistence of chronic asthma (40). Alternatively, skewing toward the Th2 profile with normal expression of Th1 cells seems to be associated with atopy alone (40). Whether the level of expression of IFN-γ is also important in the regulation of BHR in humans with asthma remains to be established.

In guinea pigs, it has been demonstrated that intranasal administration of IL-8 induces BHR to histamine, which is associated with recruitment of neutrophils to the airways (91). Additional administration of an LTB4 antagonist inhibited both the development of BHR and accumulation of neutrophils in the airways, whereas a neutrophil elastase inhibitor did not influence either of these (92), suggesting that neutrophil elastase may not be important in the development of BHR, at least in guinea pigs.

Furthermore, in mice overexpressing IL-13, features characteristic for emphysema were observed that seemed to be regulated primarily through the induction of proteolytic enzymes, including matrix metalloproteinases and cathepsins, by IL-13 (93). The involvement of IL-13 in BHR in COPD is not clear. In smokers, rapid decline in FEV1 is associated with a polymorphism in the IL-4 receptor α gene, but not with polymorphisms in the IL-13 gene, suggesting that IL-4 rather than IL-13 is involved in progression of COPD (94). Finally, CD8+ T lymphocytes are involved in the pathogenesis of COPD. IFN-γ, a product of CD8+ T lymphocytes, induces matrix metalloproteinase-12 (macrophage elastase) and cathepsin secretion, thereby shifting the pulmonary protease/antiprotease balance to favor proteolysis. This is further enhanced by inhibition of secretory leukocyte protease inhibitor by IFN-γ, thereby inducing emphysema (95). Although the effect of protease/antiprotease imbalance on BHR is unknown, one could speculate that such an enzyme imbalance may secondarily lead to BHR in subjects with emphysema because of reduced airway caliber and reduced load opposing ASM contraction.

**ASM AND BHR**

When considering BHR in relationship to asthma and COPD, one can also question whether the properties of ASM cells are different between subjects with asthma and COPD. The ASM mass in subjects with asthma and COPD is increased as compared with that in healthy individuals, although to a greater extent in subjects with asthma than in COPD (96). This increase in ASM
mass leads to changes in the thickness of the airway wall, and from this, its effects on BHR can mathematically be modeled (97). Furthermore, there are newer concepts to explain how ASM and increased mass might affect BHR. In addition to the ASM mass, the interaction between ASM cells and surrounding structures such as constituents of the airway wall and lung parenchyma is considered an important feature in BHR (97). Recently, it was demonstrated that BHR in vivo is associated with proteoglycan production by fibroblasts ex vivo (98). Moreover, a substrate of basement membrane proteins seems to stimulate the expression in vitro of a differentiated contractile phenotype and retards proliferation. On the other hand, interstitial extracellular matrix proteins promote the expression of a less contractile phenotype that is more responsive to the effects of smooth muscle mitogens (99). These studies suggest that interactions between the extracellular matrix and ASM are important in BHR. ASM cells obtained from subjects with asthma proliferate faster in culture than those obtained from patients without asthma (100). The different growth pattern of smooth muscle cells may contribute directly to the increased ASM mass in subjects with asthma and/or may be directly associated with increased responsiveness of these cells. Furthermore, cytokines in the surrounding tissue interact directly with ASM and cause decreased responsiveness to β₂-adrenoceptor agonists, stimulate cytokine secretion, inhibit or promote ASM proliferation, and “prime” ASM to become hyperresponsive to bronchoconstrictor stimuli (101).

### INTRACELLULAR MECHANISMS OF ASM CELLS AND BHR

The contractile apparatus of ASM cells consists of actin and myosin filaments, which can attach to and slide along each other (Figure 3). The light chains of myosin can interact with actin, only on their phosphorylation by myosin light chain kinase. The number of actin-myosin cross-bridges determines the force of contraction. The actomyosin cycling rate determines the rate of contraction of ASM and has been associated with BHR.

The actin–myosin cycling rate is influenced by several phosphorylation mechanisms, including that by Ca²⁺/calmodulin-dependent myosin light chain kinase, phosphorylation mechanisms independent from Ca²⁺/calmodulin, and p160 Rho kinase. The latter phosphorylates the myosin-binding subunit of myosin light chain phosphatase, thereby reducing its enzymatic activity and leading to an increased duration of light chain phosphorylation. In patients with asthma, the levels of myosin light chain kinase are believed to be upregulated, resulting in increased phosphorylation of myosin light chains, and thereby possibly directly contributing to BHR by an “endogenous” smooth muscle mechanism (102, 103).

### ICS TREATMENT AND BHR IN ASTHMA AND COPD

Currently, most patients with asthma are treated with ICS (27). In patients with COPD, treatment usually consists of symptom alleviation with bronchodilators, but frequently, ICS are also given (53). What are the consequences of ICS treatment with respect to BHR in both airway diseases?

Within weeks after the initiation of treatment with ICS, BHR to histamine or methacholine improves in patients with asthma (104–106), even with low doses of steroid (107). BHR to AMP improves to an even greater extent than that to methacholine or histamine during steroid treatment (105, 108). A recent meta-analysis has shown that there is weak relationship between the dose of beclomethasone and effect on BHR to histamine in subjects with asthma, with higher doses of beclomethasone having a more pronounced effect on BHR (109), although this could not be confirmed by others (110). A clearer dose–response relationship has been demonstrated for the dose of ICS and BHR to AMP (111).

The onset of effect of steroids on BHR seems different between AMP, histamine, and methacholine. A single high dose of ICS significantly improves BHR to AMP (112). BHR to histamine improves 72 hours after initiation of ICS treatment (113), whereas 4 weeks of treatment are needed to improve methacholine responsiveness (105). Conversely, 1 week after withdrawal of ICS, BHR to AMP reverts to pretreatment values (105), whereas such an effect occurs 2 weeks after ICS tapering for BHR to histamine (113) and methacholine (105). In addition, it has been demonstrated that within several hours of a high dose of budesonide, sputum eosinophil numbers decrease (114). This fits in with the observation that BHR to AMP is more closely associated with inflammation in the airways than BHR to methacholine (76) and may partly explain the differences in time of onset of effect on BHR.

In contrast to subjects with asthma, treatment of patients with COPD who have BHR for a period of 6 weeks with ICS does not affect BHR to methacholine or AMP (115). This lack of effect does not appear to be due to the duration of treatment because extended treatment with ICS for 6 months also did not affect BHR to histamine in patients with COPD (116). In addition, the dose of ICS was relatively high in both studies, 1,600 μg of budesonide daily in the study by Rutgers and colleagues (115) and 1,000 μg of fluticasone daily in the study by Verhoeven and colleagues (116) and is therefore not a likely explanation for these findings.

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**Figure 3.** Modulators of the rate of contraction of smooth muscle cells. After phosphorylation of the myosin light chains by myosin light chain kinase (MLCK), myosin light chains can interact with actin. The cycling rate of actin–myosin cross-bridges determines the rate of contraction of smooth muscle cells, thus contributing to BHR. MLCK is negatively regulated through protein kinase A (PKA) activity, which in turn depends on cAMP levels. Inflammatory processes result in, for instance, extracellular signal-regulated kinase (ERK) activation, which activates MLCK through interaction with regulatory proteins such as caldesmon. This will also contribute to hyperresponsiveness. ASM = airway smooth muscle. (Adapted by permission from Anderson and Rabe [102].)
The different effects of ICS treatment on BHR in asthma versus COPD might, at least in part, be explained by differences in pulmonary inflammatory profiles. As mentioned previously here, airways inflammation in asthma is characterized by eosinophilia in the lamina propria (63, 64) and induced sputum (65). In particular, the number of eosinophils in the airways is associated with the level of BHR in subjects with asthma (73, 75). After treatment with ICS in these patients, the numbers of tissue and sputum eosinophils decrease substantially in parallel with an improvement in BHR (104, 106). In addition, in patients with asthma with predominant sputum neutrophilia, BHR does not improve significantly after ICS treatment (117), suggesting an important role for eosinophils in developing and/or maintaining BHR in asthma. Indeed, when adjustment of ICS treatment is specifically aimed at normalization of sputum eosinophil numbers, there is a further improvement in BHR as compared with subjects who are treated according to conventional guidelines (118). Finally, the reduction in eosinophil number in sputum during ICS treatment is more closely related to the improvement in BHR to AMP than BHR to methacholine, suggesting that BHR testing with AMP is a more powerful instrument to monitor changes in airway inflammation in asthma (119).

Less is known about any interactions between ICS treatment, BHR, and inflammation in subjects with COPD. Inflammation in the large airways of subjects with COPD is characterized by increased numbers of CD8+ T cells and macrophages (120). In particular, the number of CD8+ T cells and also neutrophils and eosinophils in the lamina propria is inversely associated with lung function (120). Regardless, it is unknown whether inflammatory cell numbers are also associated with BHR to histamine or methacholine. ICS treatment in COPD patients initially slightly improves the level but not the rate of decline in lung function (121). In addition, such therapy does not change inflammatory cell numbers in the lamina propria of these patients (116), except for slight reductions in mast cell numbers (122). It may, therefore, not be surprising that indices of BHR to histamine also do not change during ICS treatment in patients with COPD (116). Interestingly, even though neither BHR nor FEV1 improve during short-term treatment with ICS, the small changes in these parameters appear to be positively associated (123). Finally, although AMP-induced bronchoconstriction is mediated mainly through the products of activated mast cells and basophils (11), the reductions in mast cell numbers in the airway wall mucosa, as induced by ICS treatment (122), apparently are too small to result in changes in BHR to AMP in COPD subjects (115).

**NOVEL THERAPEUTIC INTERVENTIONS IN RELATIONSHIP TO BHR**

An approach to unraveling the mechanism of BHR in asthma and COPD is in targeted intervention studies. Administration of an anti-IgE to subjects with mild asthma shows that BHR improves during prolonged treatment (124). Such a therapy also attenuates the early and late asthmatic responses to allergen (124, 125) and prevents the allergen-induced increase in BHR (125), indicating that IgE is important in the induction and maintenance of BHR in asthma.

IL-5 is a key cytokine in eosinophil differentiation and maturation in bone marrow and is involved in recruitment and activation of eosinophils toward sites of allergic inflammation (126). Blocking IL-5 with monoclonal antibodies in subjects with mild asthma completely abolishes eosinophils in peripheral blood, whereas such treatment reduces eosinophils in the bone marrow, bronchial mucosa, and bronchoalveolar lavage fluid markedly (127). Interestingly, BHR does not change during anti–IL-5 treatment in these subjects with mild asthma (127). Moreover, although blockade of IL-5 almost completely attenuates the allergen-induced increase in sputum eosinophils, the parallel increase in BHR is not affected in patients with asthma (128). These studies question the role of eosinophils in BHR.

In addition to antibodies against IL-5, several different monoclonal antibodies are now available for human use in vivo to block CD4+ T lymphocytes or IL-4. Several (pre)clinical studies with these compounds have shown promising results (129–131). So far, however, BHR has not been examined after administration of such agents to subjects with asthma. Clinical studies with monoclonal antibodies against CD4+ cells and IL-4 are awaited to examine the specific role of these factors in BHR in asthma.

A recent analysis of the Lung Health Study has shown that BHR increases in COPD subjects over time and that the increase in BHR was most pronounced in continuous smokers and those with the largest declines in FEV1 (57). Both smoking status and a decline in FEV1 were independent predictors of worsening of BHR. However, ipratropium treatment was not associated with a beneficial effect on BHR (57). At the same time, smoking cessation is associated with beneficial effects on the decline in FEV1 (132), but not with a change in inflammatory cells in induced sputum (133) or bronchial biopsy specimens (134).

A promising new therapy for COPD may be specific inhibition of phosphodiesterase-4. Recently, it was demonstrated that the number of inflammatory cells in the airway wall mucosa of subjects with COPD can be reduced by approximately 50% during treatment with cilomilast, a novel phosphodiesterase-4 inhibitor (135). If BHR is also determined by inflammatory profile in COPD, inhibition of phosphodiesterase-4 may change the level of BHR in these subjects.

**CONCLUSIONS**

What is the role of BHR in asthma? Certainly, BHR is an important feature of asthma, although not occurring in all subjects. BHR is clearly triggered by allergen inhalation, but also nonspecifically by virus infections, and is associated with the influx of inflammatory cells. Conversely, after treatment with ICS, BHR improves in conjunction with an attenuation of airways inflammation. The precise mechanisms involved in BHR are now being explored. Important Th2 cytokines associated with worsening of BHR in animal models are IL-4, IL-5, and IL-13. However, specific interventions to block each of these cytokines in humans with asthma may not per se be effective in improving BHR, as exemplified in the IL-5 blocking studies discussed previously here.

What is the role of BHR in subjects with COPD? When reviewing the current literature on BHR in COPD, it appears that even though BHR is frequently present in this patient group, it is currently a relatively underrepresented area of research and is only poorly understood. The risk factors for the development of BHR are identical to those for the development of COPD, which makes it difficult to prove whether BHR is a specific feature of COPD or is an epiphenomenon in this disorder. However, there is accumulating evidence that BHR in COPD patients is not merely a reflection of chronically reduced airway caliber but may also represent a pathophysiologic abnormality that contributes to the phenotype, at least in some patients with COPD.

The currently available and limited literature suggests that BHR in asthma and COPD are indeed different. One of the main confounding factors of BHR in COPD is baseline lung function because this may explain much of the variation in BHR. Future cellular, biochemical, and molecular studies examining alterations in ASM cell function in BHR in COPD and asthma will be helpful in increasing our understanding of the underlying mechanisms of BHR in these disorders. Given the appreciation that BHR in subjects with asthma may serve as a guiding tool
to adjust treatment, it appears desirable to understand the role of BHR in COPD and whether it represents a specific target for future therapeutic intervention.

References


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