

Provocation with adenosine 5'-monophosphate as a marker of inflammation in asthma, allergic rhinitis and chronic obstructive pulmonary disease

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Introduction

Bronchial hyper-responsiveness (BHR) and airway inflammation are both characteristic features of asthma. Histamine and methacholine, stimuli that mainly act directly on airway smooth muscle, are generally used to measure the presence and severity of BHR. Although the presence and severity of BHR are related to smooth muscle contraction, airway inflammation also contributes to BHR [1]. Therefore, provocation with an indirect stimulus that exerts its effect mainly on 'primed' cells, thereby indirectly leading to smooth muscle contraction, may provide additional information about the present inflammatory cell numbers and cellular activation state in asthma. Adenosine is such a stimulus as it acts mainly via the release of inflammatory mediators from immunologically 'primed' mast cells.

Adenosine elicits a concentration-related bronchoconstriction in asthmatic, but not in normal, individuals when administered by inhalation [2]. Commonly, the related nucleotide adenosine 5'-monophosphate (AMP) is used for practical purposes as it has much greater water solubility. Once inhaled, AMP is rapidly broken down to adenosine by the ubiquitous enzyme 5'-nucleotidase. The time to response of the airways after inhalation of AMP, and by implication adenosine, is similar to the rapid bronchoconstriction seen after histamine or methacholine challenge [3].

At this time, there is an increasing interest in the role of adenosine as a bronchoconstrictor stimulus, as it has been suggested that provocation with adenosine may be used as a non-invasive marker of airway inflammation [4,5]. The aim of the present paper is to review the literature assessing the value of AMP provocation as a diagnostic test in asthma, allergic rhinitis and chronic obstructive pulmonary disease (COPD).

The mechanism of action by which adenosine induces bronchoconstriction

Although the exact mechanism by which adenosine exerts its effect has not yet been fully elucidated, there are several lines of evidence indicating that its main mechanism of action involves the release of inflammatory mediators from mast cells. First, adenosine enhances the release of a variety of inflammatory mediators like histamine, prostaglandins, leukotrienes and IL-8 from human mast cells *in vitro* [6,7]. Secondly, it has been

found that endobronchial instillation of AMP results in an immediate increase in the level of both histamine and the (mast cell-specific) protease tryptase in bronchoalveolar lavage fluid [8–10]. The release of histamine is likely to be mast cell-rather than basophil derived, as sodium cromoglycate and albuterol have been shown to effectively inhibit AMP-induced bronchoconstriction, but have no activity against histamine release from basophils [11–13]. Thirdly, the potent and selective histamine receptor-antagonist terfenadine and astemizole have been shown to inhibit the bronchoconstrictor response to adenosine by more than 80%, suggesting that histamine is the predominant mast cell mediator involved in the airway response to this nucleotide [14–16].

Enhancement of mast cell mediator release, although prominent, may not be the only mechanism of action of AMP. Activation of neural pathways may also contribute to the bronchoconstrictor response to adenosine.

A role for reflex cholinergic vagal pathways has been suggested through the induction of pain by adenosine in human skin [17]. In a later study, the involvement of a reflex cholinergic mechanism was made unlikely by the observation that the anticholinergic agent, ipratropium bromide, at an inhaled dose sufficient to produce a 196-fold protection against methacholine, produced no significant protection against adenosine-induced bronchoconstriction [18]. There is controversy in this area, as two further studies demonstrated some protective effect [19,20]. However, in the latter two studies the protective effect of ipratropium bromide on adenosine-induced bronchoconstriction was only small and was similar for histamine. Therefore the results may well have been due to functional antagonism secondary to its bronchodilator effect.

It has also been suggested that adenosine exerts an effect via contractile neuropeptides from sensory nerve endings [21]. In support of this view, it has been demonstrated that repeated bronchial challenges with inhaled bradykinin lead to a rapid loss of the bronchoconstrictor response to AMP [22]. However, inhibition of the enzyme neutral endopeptidase, which blocks the bronchoconstrictor effects of a variety of neuropeptides, did not enhance the bronchoconstrictor effects of AMP [23]. This makes it unlikely that the release of endogenous neuropeptides contributes to the bronchoconstrictor response of adenosine.

Adenosine receptors

At least four different adenosine receptors have been identified, namely adenosine A₁, A_{2A}, A_{2B} and A₃ receptors. The rank order of potencies for various different adenosine antagonists argues against involvement of A₁, A_{2A} and A₃ receptors and is

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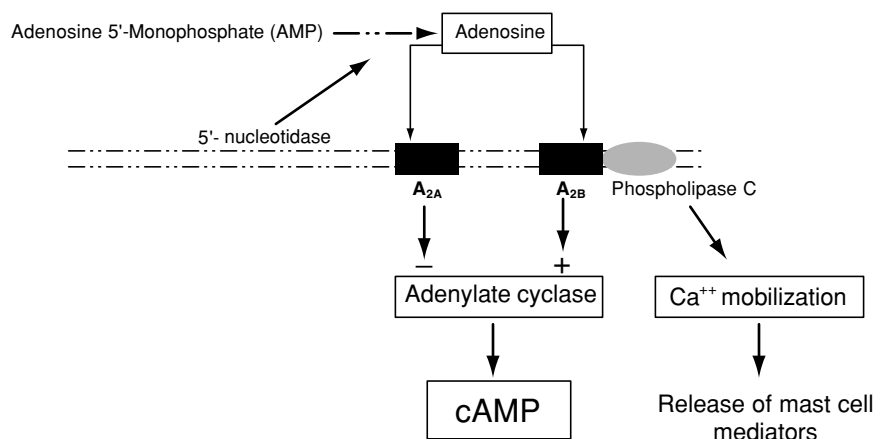


Fig. 1. Mechanism of action by which adenosine interacts with human airway mast cells, thereby eliciting the release of mast cell-derived inflammatory mediators.

entirely consistent with mediation by the A_{2B} receptor [24]. It seems unlikely that the adenylate cyclase-cAMP pathway is responsible for mast cell mediator release, as this mediator release is not inhibited by treatment of mast cells with KT5720, which completely blocks cAMP-dependent protein kinase activity [25]. Alternatively, activation of phospholipase C and the subsequent increase in intracellular calcium levels may mediate the effect of adenosine (see Fig. 1) [25].

Once inhaled, adenosine can also act on A_1 , A_{2A} and A_3 receptors, which have been identified on neutrophils, eosinophils and macrophages. Their activation causes various pro- and anti-inflammatory actions: activation of adenosine A_1 receptors promotes chemotaxis of neutrophils and increases adherence of neutrophils to endothelial cells [26–28]. By contrast, activation of adenosine A_{2A} receptors reduces chemotaxis, activation and degranulation of neutrophils [27, 29, 30]. In addition, activation of A_{2A} receptors (in contrast to activation of A_{2B} receptors) suppresses the release of tryptase from mast cells [31]. A_3 receptors mediate inhibition of eosinophil chemotaxis when activated [32]. Other functions of A_3 receptors involve inhibition of neutrophil degranulation induced by LPS or TNF- α .

PC₂₀ to AMP as marker of airway inflammation in asthma

The direct association between airway inflammation and bronchial hyper-responsiveness has been the subject of much controversy, with almost as many negative as positive reports in the literature [33–37]. It has been suggested in several studies that the PC₂₀ AMP is more closely associated with airway inflammation than the PC₂₀ methacholine or PC₂₀ histamine [4, 5, 38]. First, the PC₂₀ AMP improves to a larger extent after therapy with corticosteroids in asthma patients than the PC₂₀ methacholine [39–42]. Secondly, the improvement of asthma after a stay of 1 month in a hypoallergenic environment at high altitude in Davos could be measured with the PC₂₀ AMP but not the PC₂₀ methacholine [43]. Thirdly, we have demonstrated for the first time directly that the PC₂₀ AMP is closely associated with airway inflammation, as reflected by the percentage of eosinophils in sputum and blood, whereas the association between airway inflammation and the PC₂₀ methacholine was found to be only weak (see Fig. 2) [4]. More importantly, the steroid-induced improvement of PC₂₀ AMP was also more closely related to the concomitant reduction in airway

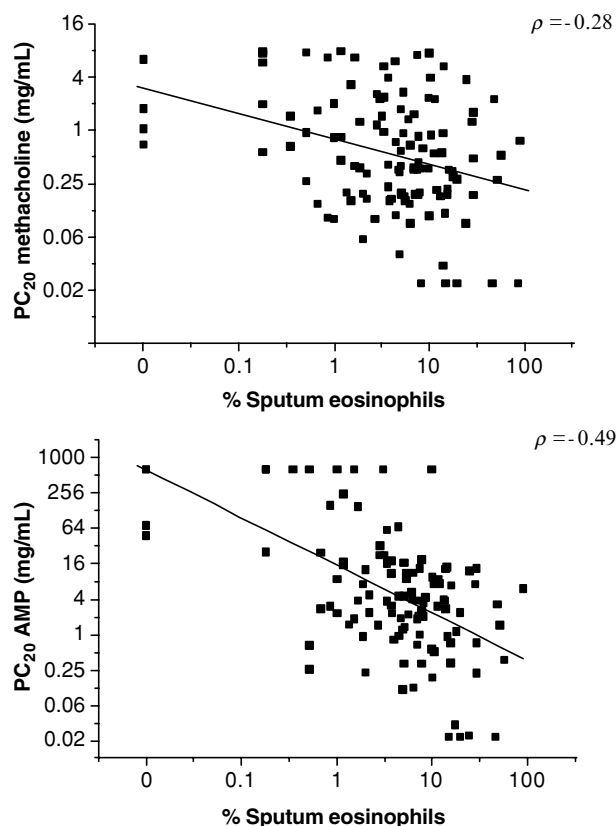


Fig. 2. Unadjusted relationship between PC₂₀ methacholine and PC₂₀ AMP with the percentage of sputum eosinophils. Adapted with permission from van den Berge et al. [4].

inflammation than the PC₂₀ methacholine [38]. Taken together, these findings indicate that the PC₂₀ AMP can be a useful tool to assess the actual inflammatory state in asthma. This may be important, as it has been shown that a treatment algorithm incorporating PC₂₀ methacholine as a surrogate marker of inflammation can improve the treatment of asthma and it suggests that monitoring with the PC₂₀ AMP may be even more appropriate for this purpose [33].

There are also drawbacks to the use of AMP challenge. A considerable percentage of patients is unresponsive to AMP [44]. The exact cut-off point for the PC₂₀ AMP is arbitrary, as

there are no population-based data on this subject. However, in a previous study by Oosterhoff et al., a value of 160 mg/mL was discriminatory between asthmatics and healthy controls [45]. Whether AMP-unresponsive patients are clinically stable (and have low levels of airway inflammation) or unstable is yet unknown. This becomes an important item when the severity of bronchial responsiveness to AMP is used to adjust treatment with corticosteroids in asthma patients [33].

To clarify this issue, we have reanalysed our own database of 120 asthma patients. Inhaled corticosteroids were tapered down until these patients developed symptoms of a pending asthma exacerbation or until they had discontinued their inhaled corticosteroids completely for 3 weeks [40]. Thereafter, all patients received treatment with corticosteroids for 2 weeks. Before treatment with corticosteroids, by design, no patient was unresponsive to methacholine ($PC_{20} > 8$ mg/mL), whereas 13 (11%) patients were unresponsive to AMP ($PC_{20} > 160$ mg/mL). After treatment with corticosteroids, 14 (12%) patients were unresponsive to methacholine and 46 (40%) patients were unresponsive to AMP. We found that patients who were unresponsive to AMP before treatment with corticosteroids had lower levels of airway inflammation than those who were responsive, their median percentage of sputum eosinophils being 0.33% and 6%, respectively (see Table 1). In addition, only two of the 13 AMP-unresponsive patients had more than 3% eosinophils in their sputum, which is considered the upper limit of normal [46]. Furthermore, after treatment with corticosteroids, AMP-unresponsive patients had also somewhat less extensive inflammation, as reflected by eosinophils and ECP in sputum and blood, than methacholine-unresponsive patients (see Table 1). Thus, this analysis supports the idea that a treatment algorithm incorporating the severity of PC_{20} AMP could possibly improve the treatment of asthma, although this has to be evaluated prospectively.

Mast cells

A possible explanation for the finding that the PC_{20} AMP is more closely associated with airway inflammation than the PC_{20} methacholine may be as follows. An overall increased

inflammatory cell activity, i.e. 'primed cells' can result in an increased production of cytokines and other mediators that increase the number and activation state of mast cells (see Fig. 3). As a result more mast cell mediators will be released after provocation with AMP, but not with methacholine. Alternatively, the number or activation state of eosinophils may be raised as a result of an increased production of inflammatory mediators by mast cells, especially IL-5.

It is unclear whether the increase in the number and activation state of mast cells, which has consistently been reported in asthma, is a feature of allergy or of the asthmatic inflammatory process itself, as allergen exposure can increase the number and activation state of airway mast cells [47]. The finding that non-allergic patients with asthma also have a bronchoconstrictor response to AMP suggests that mast cell activation can also be a feature of the asthmatic inflammatory process, rather than being dependent on the presence of allergy alone [14, 15]. Indeed, both the number and activation state of mast cells were found to be increased in non-allergic patients with asthma and therefore mast cell activation and proliferation can occur under other circumstances than allergen exposure [48–50]. It has been demonstrated *in vitro* that growth factors for human mast cells include GM-CSF, IL-4, IL-5, IL-9, IL-10, nerve growth factor and stem cell factor [51, 52]. An increased local production of one or more of these growth factors could be responsible for mast cell accumulation and activation in the airway mucosa (see Fig. 3). Nevertheless, the number of mast cells is higher in allergic than in non-allergic asthma, indicating an additive effect of allergy on mast cell counts [48]. In agreement with this, inhaled adenosine was found to be three times more potent in patients with allergic asthma than in patients with non-allergic asthma, whereas the degree of bronchial hyper-responsiveness to a direct stimulus did not differ between both groups [2, 53].

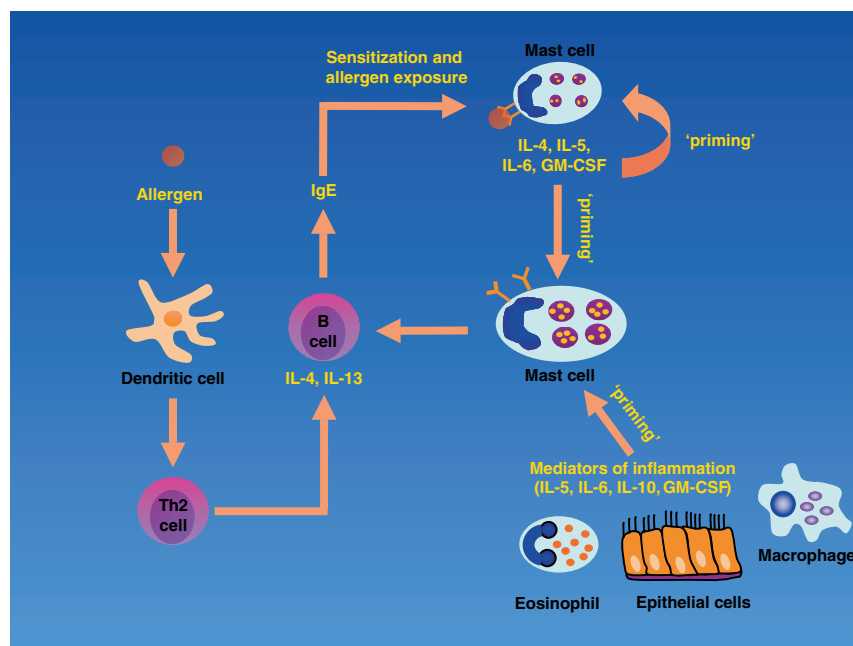
In recent years, there has been a tendency to attach less importance to the role of the mast cell as an effector cell in asthma. However, new findings have led to a resurgence of interest in the mast cell and support a re-evaluation of its role. In particular, the discovery that mast cells are a source of cytokines has suggested new ways in which mast cell activation could also participate in more persistent and even chronic inflammatory responses [54–56]. For example, it has been

Table 1. Comparison of the level of lung function (FEV_1) and airway inflammation (i.e. eosinophils and ECP in sputum and blood) in patients who are unresponsive to methacholine or to AMP, before and after treatment with corticosteroids*

	Before steroid treatment			After steroid treatment			
	PC ₂₀ MCh	PC ₂₀ AMP		PC ₂₀ MCh		PC ₂₀ AMP	
	≤ 8 mg/mL <i>n</i> = 120	≤ 160 mg/mL <i>n</i> = 101	>160 mg/mL <i>n</i> = 13	≤ 8 mg/mL <i>n</i> = 99	>8 (mg/mL) <i>n</i> = 14	≤ 160 (mg/mL) <i>n</i> = 67	>160 mg/L <i>n</i> = 46
FEV ₁ (%pred)	78 (77–92)	79	92 (87–99)	86 (74–97)	92 (69–101)	83 (70–96)	91 (80–103)
<i>Sputum</i>							
Eosinophils (%)	5 (2–11)	6 (3–13)	0.33 (0.17–1.3)	0.5 (0–2.3)	0.3 (0–1.3)	1 (0.2–4.5)	0.17 (0–0.8)
≥ 3% eosinophils, <i>n</i> (%)	100 (83)	72 (73)	2 (15)	29 (30)	2 (14)	20 (31)	2 (4)
ECP (ng/L)	38 (17–125)	52 (21–127)	13.6 (4.5–30)	16 (7–47)	47 (12–102)	22 (9–57)	16 (6.4–44)
<i>Blood</i>							
Eosinophils (10 ⁹ /L)	0.37 (0.22–0.5)	0.35 (0.25–0.5)	0.16 (0.1–0.36)	0.26 (0.12–0.36)	0.2 (0.1–0.27)	0.22 (0.14–0.35)	0.18 (0.11–0.27)
ECP (ng/L)	38 (17–125)	17 (10–25)	10.4 (6.1–18)	10 (6–17)	10 (6–44)	10 (6–18)	9.5 (7.4–16)

*Values are expressed as median with interquartile ranges between brackets, unless otherwise specified.

Fig. 3. Schematic diagram of the mechanisms involved in mast cell priming. The mast cell can be primed by a vast array of inflammatory mediators, which can be produced during both allergic (IgE mediated) and non-allergic processes (release of inflammatory mediators during an overall increased inflammatory activity). In turn the mast is capable of influencing IgE secretion from B cells, thereby further aggravating the inflammatory process in asthma.



demonstrated that the ability to produce IL-4 and IL-13 enables mast cells to stimulate IgE secretion by B cells (see Fig. 3) [57–59]. This may explain the finding that, although the concentration of serum total IgE falls within the normal range in patients with intrinsic asthma, it is still higher than in non-allergic subjects without asthma [60]. In support of the notion that mast cells can contribute to IgE concentrations in intrinsic asthma, it was found that the level of IL-4 is elevated in these patients and (as in allergic asthma) that mast cells express a significant proportion of this cytokine [61–63]. Interestingly, a higher concentration of serum total IgE is associated with a lower lung function in asthma patients, irrespective of atopic status [2]. Taken together, these observations have obvious implications for the role of mast cells in both allergic and intrinsic asthma. Provocation with AMP may therefore become a useful tool for further exploration of the role of mast cells in asthma, as there is now convincing evidence that the airway response to this nucleotide is an index of mast cell number or activity.

Allergic rhinitis

Polosa and colleagues recently demonstrated, similar to the findings in asthma patients, that the PC₂₀ AMP, but not the PC₂₀ methacholine, was associated with lower airway inflammation in subjects with allergic rhinitis in whom the diagnosis of asthma was specifically excluded [5]. Although asthma and allergic rhinitis are different diseases, there are strong clinical and epidemiological associations between allergic rhinitis and extrinsic asthma. Allergic rhinitis often precedes the onset of clinical asthma and is a risk factor for the development of asthma [64,65]. In addition, hyper-responsiveness can be present in subjects with allergic rhinitis without clinical evidence of asthma [5,66]. Little is known about the factors that determine bronchial hyper-responsiveness in these subjects, but it may be due to an increased inflammatory activation state (i.e. an increase of eosinophils in biopsies and induced sputum) within the

airways of these subjects [37,67,68]. It has been demonstrated in an epidemiological study that the presence of an increased inflammatory activation state, as reflected by the presence of both peripheral blood eosinophilia and bronchial hyper-responsiveness, increases the risk of developing asthma [69]. In this respect, the observation of Polosa and colleagues that a higher level of bronchial hyper-responsiveness to AMP is associated with an increase in the inflammatory activation state in the airways of non-asthmatic subjects with allergic rhinitis is of particular interest, as it may indicate a predisposition to develop asthma. However, this hypothesis has to be confirmed in long-term prospective studies of patients with allergic rhinitis, including assessment of sputum eosinophilia and bronchial hyper-responsiveness to both AMP and methacholine.

COPD

AMP can induce bronchoconstriction in subjects with COPD as well. Airway inflammation may play a role in AMP-induced bronchoconstriction, as hyper-responsiveness to AMP has a higher prevalence and severity in smokers than in non-smokers (or ex-smokers) with COPD who have comparable levels of hyper-responsiveness to methacholine (see Fig. 4) [70]. This is in agreement with findings of increased concentrations of trypsinase and histamine in the bronchoalveolar lavage fluid of smokers [71,72]. Although mast cells are commonly not associated with the inflammatory process in COPD, higher numbers of mast cells have been found by several groups [71,72]. It has recently been demonstrated in patients with COPD that the presence of hyper-responsiveness to AMP is accompanied by higher percentages of sputum eosinophils [73]. Airway inflammation can contribute to airflow limitation in a subgroup of patients with COPD and the presence of eosinophilic inflammation can predict benefit from corticosteroids [74,75]. Thus, it can be hypothesized that the identification of eosinophilic airway inflammation in COPD via hyper-responsiveness to

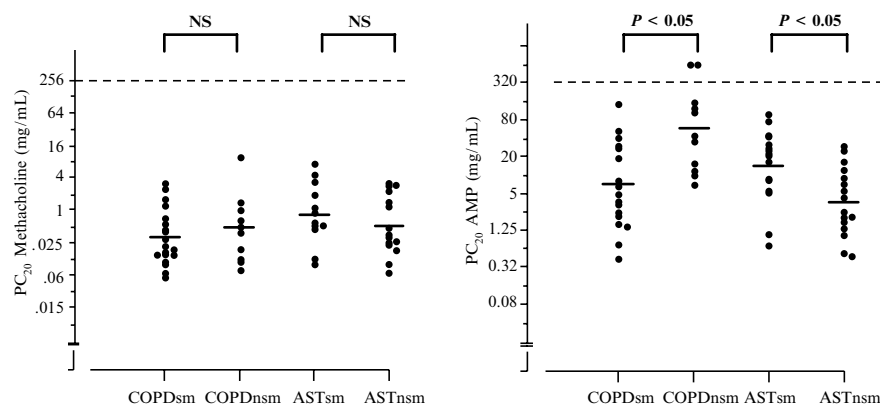


Fig. 4. PC₂₀ methacholine (left) and PC₂₀ AMP (right) values of smoking subjects with COPD (COPDsm), non-smoking subjects with COPD (COPDnsm), smoking asthmatics (ASTsm) and non-smoking asthmatics (ASTnsm). Individual values are presented as closed circles. Mean values, expressed as geometric mean, are represented by horizontal bars. Dashed line indicates the highest value administered. Compiled with permission from Oosterhoff et al. [45,79].

AMP creates therapeutic possibilities. In support of this, the improvement of PC₂₀ AMP after 6 weeks of treatment with a high dose of inhaled corticosteroids was significantly correlated with the number of peripheral blood eosinophils at baseline [73].

In patients with COPD, Renkema and colleagues recently found that a higher concentration of serum total IgE (indicating an increased inflammatory state) is associated with a lower decline in PC₂₀ histamine over time [76]. This was a surprising finding, as only non-allergic patients without symptoms or signs of asthma were entered into this study. In the absence of allergy, it can be speculated that mast cells contribute to the serum IgE level, as they have the potential to influence IgE secretion from B cells and they are present in increased numbers in the airways of COPD patients [58,59,71]. If so, the increased serum IgE level in a subgroup of COPD patients might also be related to the severity of bronchial responsiveness to AMP. It can be inferred from the above that a provocation test with AMP might provide different information about the prognosis in COPD patients. It is now generally accepted that a more severe bronchial responsiveness to methacholine or histamine is associated with a poorer prognosis (i.e. faster decline in FEV₁) in COPD patients [77,78]. In contrast, a more severe bronchial responsiveness to AMP might be related to a more favourable prognosis, although this has yet to be determined.

It has been suggested that provocation with AMP can be useful as a test to differentiate between asthma and COPD. This was based on the finding that ex-smokers with COPD (but not smokers with COPD) are significantly less responsive to AMP than non-smoking asthmatic subjects, whereas the sensitivity to a direct stimulus was the same between both groups [79]. However, in this study only non-smoking asthmatics were selected and this may have lowered the PC₂₀ value in the group of asthma patients, since non-smoking asthmatics are possibly less responsive to AMP due to a 'healthy smoker' effect (see Fig. 4) [45]. In addition, patients were not allowed to take inhaled corticosteroids. This may have increased the sensitivity to AMP to a greater extent in the patients with asthma than (if at all) in the patients with COPD. Thus we feel that it is, at present, premature to suggest a role for provocation with AMP in differentiating between asthma and COPD.

Conclusion

AMP is an indirect stimulus to measure bronchial hyper-responsiveness, as it mainly acts via the release of inflammatory

mediators from mast cells. The PC₂₀ AMP is more closely associated with airway inflammation than PC₂₀ methacholine in asthma. In addition, the PC₂₀ AMP more closely reflects changes in airway inflammation than PC₂₀ methacholine, either after treatment with corticosteroids or after a stay in a hypoallergenic environment. Thus, PC₂₀ AMP may be a better tool to monitor airway inflammation in asthma. In addition, the PC₂₀ AMP is associated with inflammation in the lower airways of patients with allergic rhinitis and without a clinical history of asthma. AMP can induce bronchoconstriction in subjects with COPD as well and in agreement with this observation higher numbers of mast cells have been found in the airways of these patients. Finally, it has been suggested that a management plan incorporating the PC₂₀ AMP might improve the treatment of asthma. This hypothesis has to be sorted out in future studies.

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