Adenosine 5′-monophosphate challenge elicits a more peripheral airway response than methacholine challenge

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Michils A, Elkrim Y, Haccuria A, Van Muylem A. Adenosine 5′-monophosphate (AMP) and methacholine are commonly used to assess airway hyperreactivity. However, it is not fully known whether the site of airway constriction primarily involved during challenges with either agent is similar. Using a ventilation distribution test, we investigated whether the constriction induced by each agent involves the lung periphery in a similar fashion. Ventilation distribution was evaluated by the phase III slope (S) of the single-breath washout, using gases with different diffusivities like helium (He) and hexafluorosulfur (SF6). A greater postchallenge increase in SHe reflects alterations at the level of terminal and respiratory bronchioles, while a greater increase in SSSF6 reflects alterations in alveolar ducts, increases to an equal extent reflecting alterations in more proximal airways where gas transport is still convective for both gases. SSSF6 and SHe were measured in 15 asthma patients before and after airway challenges (20% forced expired volume in 1 s (FEV1) with AMP and methacholine. SHe increased to a greater extent than SSSF6 after AMP challenge (5.7 vs. 3.7%/l; P = 0.002), with both slopes increasing to an equal extent after methacholine challenge (3.1%/l; P = 0.959). The larger increase in SHe following AMP challenge suggests distal ventilation impairment up to the level of terminal and respiratory bronchioles. With methacholine, the similar increases in SHe and SSSF6 suggest a less distal impairment. AMP, therefore, seems to affect more extensively the very peripheral airways, whereas methacholine seems to have an effect on less distal airways.

asthma; airway hyperresponsiveness; methacholine; ventilation distribution; helium; hexafluorosulfur

Adenosine 5′-monophosphate (AMP) and methacholine are commonly used to assess airway hyperreactivity (AHR). AHR is a defining feature of asthma, reflecting the increased sensitivity of asthmatic airways to a range of inhaled stimuli (18). It can be measured by direct or indirect challenge tests, the difference referring to the mechanism by which the bronchoconstricting agent elicits a bronchospasm (19, 30). Methacholine acts directly on smooth muscle cells (30), whereas AMP acts indirectly by activating different cell types, including primed mast cells, which in turn result in a release of bronchoconstrictor mediators, as well as neural pathways (19, 38). It is generally believed that challenges with AMP and methacholine may reveal different components of AHR indicating that it is more appropriate to refer to the agent used to assess AHR, thus making the distinction between AHR to AMP and AHR to methacholine (2, 8). Indeed, the provocative concentration (PC) causing a 20% fall in forced expired volume in 1 s (FEV1) for AMP (PC20 AMP) is more sensitive to airway inflammation than is PC20 methacholine (32, 33), which appears more determined by long-term changes in airway structure (12, 29, 31, 39).

In addition, because receptor sites for each agent are not evenly distributed throughout the lung, one could expect a difference regarding the location of airway constrictions induced by each agent. Autoradiographic mapping has shown that, in humans, receptors interacting with methacholine are localized to smooth muscles of all airways with a higher density in larger airways (4, 22). In contrast, the density of mast cells through which AMP acts to induce bronchospasm is higher in distal than in proximal airways (6). Therefore, we hypothesized that the bronchoconstriction induced by methacholine and AMP during airway challenge might actually occur at different sites. To determine the location of airway alteration, we performed ventilation distribution tests using inert gas before and after AMP and methacholine challenges in asthma patients.

METHODS

Subjects

Twenty patients newly diagnosed with asthma were recruited from the outpatient asthma clinic (Cliniques Universitaires de Bruxelles, Erasme University Hospital, Brussels, Belgium). Asthma was defined according to standard criteria (3). At the time of inclusion, the patients were nonsmokers and had no recent history of upper airways infection. None of them had received any asthma treatment except for on-demand short-acting inhaled β2-agonists. The study was approved by the local ethics committee (Cliniques Universitaires de Bruxelles, Erasme University Hospital), and all patients signed an informed consent.

Study Design

The study, which was designed as a prospective single-blind crossover study, was carried out in the Chest Department of Erasme University Hospital.

Patients attended the lung function laboratory on two occasions separated by ≥2 days. Ventilation distribution was evaluated by the single-breath washout method before and immediately after airway challenges, which were performed either first with AMP and then with methacholine or first with methacholine and then with AMP on a random basis. During each set of measurements, single-breath washout tests were performed after spirometry. Short-acting inhaled β2-agonists were withheld for at least 6 h before either challenge. Atopic status was evaluated by skin prick testing using common inhalant allergens during the first visit.

Patients who did not elicit AHR to both methacholine and AMP (n = 5, i.e., 4 subjects elicit AHR to methacholine only and 1 to AMP only) were excluded from the analysis.
Slopes (S) for each gas (SHe, SF6) were computed by a computerized linear regression (concentration vs. expired volume) between 35 and 80% of the expired volume. If needed, a manual setting of the regression limits allowed for avoiding dead space or closing volume. Figure 1 presents typical He, SF6 concentration tracings. He and SF6 slopes, and slope changes, were expressed in percent per liter. To be comparable with the values of nitrogen slope reported in the literature, He and SF6 slopes were multiplied by −15.6 (see APPENDIX A). The test was performed in triplicate with a variation coefficient not exceeding 10%.

Statistical Analysis

Comparisons of AMP and methacholine challenges baseline values and pre- and postchallenge values were performed using paired t-tests. Slope change comparisons between AMP and methacholine challenges, for each gas, and between He and SF6, for each challenge, were conducted using an one-way ANOVA for repeated measurements with Tukey’s post hoc comparisons. The level of significance (two-tailed) was 0.05. The Statistica 6.1 program (StatSoft France, Maisons-Alfort, France) was used for the analyses.

RESULTS

Patients Characteristics

Table 1 presents patients characteristics. As mentioned earlier, all patients enrolled in the study were corticosteroids naive. A 6-mo follow-up indicated that none of the 15 patients included in the final analysis suffered from severe asthma.

Baseline Values

Baseline percentage-predicted pulmonary function indexes and baseline ventilation distribution indexes were similar before AMP and methacholine challenges (Table 2).

PC20 and Percentage of Maximal Dose

PC20, expressed as geometric mean [geometric interval], was 0.46 [0.01–23.07] mg/ml for methacholine and 10.2 [2.8–37.8] mg/ml for AMP. If m and SD are mean and standard deviation of the PC20 logarithms, respectively, the geometric mean is computed as antilog(m) and the geometric interval as [antilog(m − SD) – antilog(m + SD)]. PC20 AMP was reached at a lower percentage of the maximal dose (2.8%, interquartile range: 1.3–9.0%) than PC20 methacholine (6.1%, interquartile range: 3.8–24.9%). These percentages were significantly different (P = 0.016, Wilcoxon test).

Pre- and Postchallenge Changes

Table 3 presents the pre- and postchallenge differences of spirometric indexes (means ± SD) in percentage of the base-line values.

Table 1. Anthropometric data and ACQ score before the challenges

<table>
<thead>
<tr>
<th>n</th>
<th>M/F</th>
<th>Age</th>
<th>ACQ score (median [interquartile range])</th>
<th>ICS use</th>
<th>Atopic status</th>
<th>Occupational asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>6/9</td>
<td>37.3 ± 13.2</td>
<td>1.4 [0.0–3.2]</td>
<td>0</td>
<td>14/15</td>
<td>0</td>
</tr>
</tbody>
</table>

Age values are means ± SD. ACQ, asthma control questionnaire. n, number; M, male; F, female; ICS, inhaled corticosteroids.

Study Procedures

Lung function. Lung function was measured using a Zan 300 spirometer (Zan, Obershulha, Germany) according to standardized guidelines (28). Values for the forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), inspired capacity (IC), and forced expired flow at 75% of maximal expired volume (FEF75) were expressed as a percentage of predicted value (%pred; Ref. 28), while changes were expressed as a percentage of the baseline value (Δ%).

AMP and methacholine challenge procedures. Bronchial challenges to AMP or methacholine were performed using the dosimeter method according to established protocols (19, 30). After an initial nebulized saline challenge, either AMP (Sigma-Aldrich, St. Louis, MO, USA) or methacholine (Sigma-Aldrich) was administered using Prov Air nebulizer (Zan, Obershulha, Germany) in increasing concentrations from 1.563 to 400 mg/ml for AMP and from 0.038 to 8 mg/ml for methacholine. The procedure was stopped when FEV1 had decreased by ≥20% from its postsaline solution inhalation value. Short-acting β2-agonist puffs (salmeterol, 400 μg) were then administered to aid recovery.

Ventilation distribution tests with inert gases. Ventilation distribution was assessed by single-breath washout tests. Subjects were connected to a double bag-in-box system through a nonrebreathing valve with a 20-ml instrumental dead space. Patients inhaled a gas mixture containing two inert gases, 5% helium (He) and 5% hexafluoro sulfur (SF6) in oxygen (O2), from functional residual capacity (FRC) to 1 liter above FRC and then expired at a constant flow of ~0.40 l/s to residual volume. This test (1 l inspired from FRC) was repeated in triplicate with a variation coefficient not exceeding 10%. During expiration, He and SF6 concentrations were recorded as a function of expired volume by a quadrupole mass spectrometer (LR6000 Logan-Sinclair, Rochester, UK) calibrated immediately before each set of measurements (calibration cylinders: 6% CO2, 15% O2, 79% N2 and 5% He-5% SF6-90% O2; Messer Belgium, Zwijndrecht, Belgium). Volume-concentration delay (due to the gas capillary transit time) was assessed when the subject re-inspired the 5% He-5% SF6-90% O2 mixture after having reached residual volume. The signals were then instantaneously synchronized.

Fig. 1. Typical baseline helium (He; solid line) and hexafluorosulfur (SF6; dashed line) concentration tracings as a function of the expired volume after 1-liter inspiration from functional residual capacity. Expiration ended at residual volume. Inspiratory concentration was 5% for both gases. Linear regression between 35 and 80% of the total expired volume are indicated by the bold straight lines.
line values. All indexes significantly changed during the challenges, but no significant differences in index changes were seen between AMP and methacholine challenges. The average decrease in inspired capacity, reflecting the increase in FRC, was equal to 300 ml during both challenges. Regarding the ventilation distribution indexes, Table 4 shows that SF6 slope increases were significant and similar after AMP and methacholine challenges, whereas He slopes increased significantly more during AMP challenges than during methacholine challenges. Moreover, during AMP challenge, He slope increased to a greater extent than that of SF6, whereas He and SF6 slopes increased to the same extent during methacholine challenge. Figure 2 allows for individual comparisons between AMP and methacholine challenges for the changes in He slopes (Fig. 2A) and SF6 (Fig. 2B). Figure 2, C and D, allows for individual comparisons between He and SF6 slopes changes during AMP and methacholine challenges, respectively.

Clinical Significance of Changes in Ventilation Distribution Indexes

In a long-term longitudinal context, the upper limit of He, SF6 slopes normality (upper limit of the 95% confidence interval) was found to be 1.6 times the baseline value (+60%; Ref. 36). This criterion is likely to be too strong for the present study, as it accounts for variation over months. On average, this criterion was met since He slopes increased by 167 and 61%, during AMP and methacholine challenges, respectively. Moreover, the terminal bronchioles where the parenchyma had been altered at the level of terminal and respiratory bronchioles. This has been shown to occur in lung transplant recipients (14, 34) when bronchiolitis obliterans developed predominantly in the terminal bronchioles area (i.e., preacinar region), whereas methacholine elicits a response in less peripheral airways. To demonstrate this, we evaluated ventilation distribution using the single-breath washout method before and after airway challenges performed with each agent. Studying ventilation distribution with inert gases may bring insight into the location of airways alterations. When a nonresident inert gas is inhaled and then expired, its concentration as a function of the expired volume reaches a plateau, the so-called “phase III,” following dead space exhalation. The slope of this plateau reflects ventilation heterogeneities generated during inspiration and sustained during expiration. When two nonresident inert gases differing only in their diffusive properties, like He and SF6, are simultaneously inhaled and expired by a normal subject, they produce slopes of different magnitudes, providing evidence for the role of molecular diffusion in the genesis of the phase III slope. Theoretical considerations, along with anatomical knowledge about bronchial tree dimensions (17, 40) have shown that the slope of a given gas is particularly sensitive to airways alterations occurring where diffusion transport balances convection transport, sometimes called “diffusion front.” By estimating axial velocity along the bronchial tree during inspiration (40) and taking into account gas diffusion coefficients, this zone may be located at the level of terminal and respiratory bronchioles for He and, deeper inside the acinus, at the level of alveolar ducts, for SF6. The impact of proximal bronchoconstriction was simulated in APPENDIX A, and it appeared that proximal bronchoconstriction had very little effect on the position of the diffusion front (Fig. 3). The latter was also shown to be insensitive to distal structural alterations (13). This permits fair comparisons of postchallenge values with baseline values and of AMP challenge with methacholine challenge in the same subject.

Given the above, when alterations to the airways are induced, three situations are likely to occur: 1) The He slope increases to a greater extent than SF6 slope, likely reflecting alterations at the level of terminal and respiratory bronchioles. This has been shown to occur in lung transplants recipients (14, 34) when bronchiolitis obliterans developed predominantly in the terminal bronchioles where the parenchyma had been preserved (1). In this context, a longitudinal study also dem-

Table 2. Baseline values of standard pulmonary function indexes in percentages predicted and of single-breath washout indexes

|                   | AMP    | Methacholine | P
|-------------------|--------|--------------|--
| FEV1, %pred       | 91.7 ± 13.8 | 96.5 ± 17.4 | 0.068
| FVC, %pred        | 99.9 ± 15.0 | 103.0 ± 14.7 | 0.051
| FEF75, %pred      | 59.2 ± 23.7 | 68.6 ± 34.7 | 0.273
| IC, %pred         | 98.5 ± 15.7 | 100.9 ± 19.0 | 0.195
| SH%, %/l          | 3.4 ± 2.0   | 3.7 ± 2.6  | 0.394
| SBF%, %/l         | 4.8 ± 2.2   | 5.1 ± 2.9  | 0.382

Values are means ± SD. AMP, adenosine 5’ monophosphate; FEV1, forced expired volume in 1 s; FVC, forced vital capacity; FEF75, forced expired flow at 75% of maximal expired volume; IC, inspired capacity; SH% and SBF%, slope of phase III of He and SF6 expired concentration tracings, respectively; P, statistical significance of AMP-methacholine difference.

Table 3. Pre- and postchallenge changes of spirometric indexes in percentage of baseline value

|                   | AMP       | Methacholine | P (pre-post) | P (AMP-Methacholine)
|-------------------|-----------|--------------|--------------|---------------------
| ΔFEV1, %baseline  | −28.1 ± 8.6 | <0.001       | −26.6 ± 7.0 | <0.001 0.494
| ΔFVC, %baseline   | −20.2 ± 13.1 | <0.001       | −15.3 ± 11.7 | <0.001 0.099
| ΔFEF75, %baseline | −28.1 ± 20.4 | <0.001       | −33.3 ± 27.6 | <0.001 0.482
| ΔIC, %baseline    | −14.7 ± 15.1 | 0.003        | −9.8 ± 13.0 | 0.012 0.263

Values are means ± SD, Δ%, percentage of baseline value; P (pre-post), statistical significance of pre- and postchallenge difference; P (AMP-Methacholine), statistical significance of AMP-methacholine difference in pre- and postchallenge differences.

DISCUSSION

Our findings indicate that, in patients suffering from moderate-to-mild allergic asthma, the mechanism by which FEV1 falls in response to AMP is different from that by which FEV1 falls in response to methacholine. Indeed, AMP elicits a response in the lung periphery including the furthest reaches of the terminal bronchioles area (i.e., preacinar region), whereas methacholine elicits a response in less peripheral airways. To demonstrate this, we evaluated ventilation distribution using the single-breath washout method before and after airway challenges performed with each agent. Studying ventilation distribution with inert gases may bring insight into the location of airways alterations. When a nonresident inert gas is inhaled and then expired, its concentration as a function of the expired volume reaches a plateau, the so-called “phase III,” following dead space exhalation. The slope of this plateau reflects ventilation heterogeneities generated during inspiration and sustained during expiration. When two nonresident inert gases differing only in their diffusive properties, like He and SF6, are simultaneously inhaled and expired by a normal subject, they produce slopes of different magnitudes, providing evidence for the role of molecular diffusion in the genesis of the phase III slope. Theoretical considerations, along with anatomical knowledge about bronchial tree dimensions (17, 40) have shown that the slope of a given gas is particularly sensitive to airways alterations occurring where diffusion transport balances convection transport, sometimes called “diffusion front.” By estimating axial velocity along the bronchial tree during inspiration (40) and taking into account gas diffusion coefficients, this zone may be located at the level of terminal and respiratory bronchioles for He and, deeper inside the acinus, at the level of alveolar ducts, for SF6. The impact of proximal bronchoconstriction was simulated in APPENDIX A, and it appeared that proximal bronchoconstriction had very little effect on the position of the diffusion front (Fig. 3). The latter was also shown to be insensitive to distal structural alterations (13). This permits fair comparisons of postchallenge values with baseline values and of AMP challenge with methacholine challenge in the same subject.

Given the above, when alterations to the airways are induced, three situations are likely to occur: 1) The He slope increases to a greater extent than SF6 slope, likely reflecting alterations at the level of terminal and respiratory bronchioles. This has been shown to occur in lung transplants recipients (14, 34) when bronchiolitis obliterans developed predominantly in the terminal bronchioles where the parenchyma had been preserved (1). In this context, a longitudinal study also dem-
onstrated the great sensitivity of the He-SF6 single-breath technique even at an early-stage of bronchiolitis obliterans (14). 2) The SF6 slope increases to a greater extent than the He slope, likely reflecting alterations inside the acinus. Such a slope-difference has been positively correlated with alterations up to alveolar ducts in chronic obstructive pulmonary disease (35). 3) The two slopes increase to an equal extent. This situation may correspond either to alterations occurring in more proximal airways, i.e., before terminal bronchioles, where the two inert gases are transported mostly by convection, or to alterations occurring at the level of terminal and respiratory bronchioles (increasing He slope) and inside the acinus (increasing SF6 slope).

When FEV1 had fallen by \( \geq 20\% \), the increases in slopes reached both statistical and clinical significances. The greater He slope increase with AMP (corresponding to the first aforementioned situation) indicated that the challenge, at that point, had impacted the bronchial tree down to the terminal bronchioles. When a similar level of constriction (i.e., \( \geq 20\% \) fall in FEV1) was induced by methacholine, He and SF6 slope increased by the same amount, corresponding to the third aforementioned situation. When compared with AMP challenge, He slopes increased less while SF6 slopes increased by

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<thead>
<tr>
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<th>AMP</th>
<th>Methacholine</th>
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<tr>
<td>( S_{He} ), %/l</td>
<td>5.7 ± 2.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( S_{SF6} ), %/l</td>
<td>3.7 ± 2.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( P(\text{He-SF6}) )</td>
<td>0.002</td>
<td>0.959</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( \Delta \), pre- and postchallenge slope change; \( P \) (pre-post), statistical significance of pre- and postchallenge difference for given gas and challenge; \( P \) (AMP-Methacholine), statistical significance of \( \Delta \) slopes difference between AMP and methacholine challenges for a given gas; \( P \) (He-SF6), statistical significance of \( \Delta \) slopes difference between He and SF6 for a given challenge.

![Fig. 2. Comparisons of individual slope changes (\( \Delta S \)): He slope changes during AMP (●) and methacholine (○) challenges; SF6 slope changes during AMP (▲) and methacholine (▼) challenges. Linked pairs of symbols represent changes in the same patient. Means ± SD are indicated with the same symbols as the individual values. A: comparison of He slope changes between AMP and methacholine challenges. B: analog to A for SF6 slope changes. C: comparison between He and SF6 slope changes during AMP challenges. D: analog to C for methacholine challenge.](image1)

![Fig. 3. Simulation of He (solid line) and SF6 (dashed line) concentration profiles as a function of the generation number, at the end of a 2-s inspiration at 500 ml/s. A: no bronchoconstriction. B: bronchial lumen cross section divided by 2 from generation 3 up to generation 14 (horizontal gray line). C: bronchial lumen cross section divided by 2 from generation 3 up to generation 17.](image2)
the same amount. This most probably suggests a less distal impact of the methacholine challenge.

A decline in FVC is usually considered as reflecting airway closure elicited by bronchoconstriction of the so-called “small airways,” namely from generation 6 to generation 19 of the bronchial tree, according to Weibel’s morphometrical data (40). The similar decline in FVC following both challenges, in line with previously reported data (9), likely indicates that both agents act on this peripheral conductive portion of the airways. This supports the findings of Manrique et al. (23) who showed similar gas exchange disturbances after challenges with methacholine and AMP, suggesting a peripheral action of both agents. It should be noted that, in the study by Manrique et al., challenges were performed until a FEV₁ fall >30% was achieved compared with 20% in our study. Although the doses were not mentioned, this deeper FEV₁ decrease (~35%) suggests the use of higher doses of both agents, which may have blurred potential differences. In addition, although FVC decrease and gas exchange disturbances may reflect impairments occurring in the lung periphery as a whole, they are unlikely to pinpoint the sites of alteration within this zone. In contrast, the He-SF₆ single-breath test used in our study allows us to bring further insight into the sites of alterations by emphasizing different patterns of slope increase that indicate distinct action sites for methacholine and AMP, the latter acting up to the furthest terminal bronchioles. It must be emphasized that the involvement, in the constriction process, of even a few more generations in the distal part of the bronchial tree implies an action on, at least, one order of magnitude greater number of bronchioles. Therefore, provocative agents like AMP, along with sensitive ventilation heterogeneity tests, might be useful tools in assessing how accurately standard spirometry captures constriction in such a vast zone.

It is interesting to note that the same ventilation distribution tests used before and after cold dry-air hyperventilation challenges, in adult (21) and children (16) asthma patients, revealed results that were qualitatively similar to those observed with AMP challenges, i.e., He slope increase greater than the SF₆ slope increase. Altogether, this suggests that a very peripheral constriction may be a general feature of airway challenges that act through mast cell activation (i.e., indirect and allergen challenges). In contrast, methacholine appears to act on less peripheral airways (20, 27).

Although the exact reasons for these different response patterns to AMP and methacholine have not yet been definitively elucidated, it seems reasonable to link them to the different receptors through which the agents interact within the airways and their respective distribution throughout the lung. More precisely, methacholine interacts with muscarinic receptors, which are localized on the smooth muscles throughout the entire airway, yet with a higher density in larger airways (4, 22). AMP challenge, in contrast, leads to the release of several bronchoconstricting mediators through the degranulation of mast cells (19, 38). The diversity of mediators and possibly the localization of their receptors in the lung as well as the greater concentration of mast cells in the distal airways, as previously reported (6), may account for the stronger peripheral impact of AMP. While this theory is sensible, this study is the first to actually support this hypothesis.

Of course, one cannot exclude the possibility that the size of aerosolized particles could also have influenced the distribution of aerosol within the bronchial tree, thus giving rise to the induction of bronchoconstriction at different locations (i.e., a more central deposition during methacholine challenge). Indeed, it has been shown recently by Cohen et al. (10) that particle size matters during airway challenges, at least with AMP: small-particle AMP induced a 20% fall in FEV₁ (PC₂₀) at a significantly higher dose than large-particle AMP and changes in AHR to small-particle AMP seemed to better reflect benefit from ultrafine inhaled corticosteroids. Given this, it is important to note that, in our study, the nebulization conditions were strictly similar with both products. Indeed, both challenge procedures were performed using the same dosimeter method (7) according to standard guidelines (19, 30) and with the same device (Prov Air nebulizer; Zan), which delivers particles with an aerodynamic mass median diameter of 5 μm at an output of 14 μl per breath. We are not aware of any influence on aerosol distribution within the bronchial tree that might result from differences in agent molecular surface tension.

Regardless of the reason for the observed differences, our results suggest that AMP challenge is more appropriate to investigate AHR of very distal airways. As mentioned earlier, PC₂₀ AMP has been shown to be more closely associated with airway inflammation (33) and to improve more rapidly and to a larger extent following inhaled corticosteroids than PC₂₀ methacholine (32). It is, therefore, tempting to speculate that the “very distal” reactivity reflected in PC₂₀ AMP, but not in PC₂₀ methacholine, could be associated with distal airway inflammation (i.e., enhanced mast cell expression and activity), which could be reversed by inhaled corticosteroids. This remains to be investigated in a prospective way. It is also unknown whether the different mechanisms of PC₂₀ AMP and PC₂₀ methacholine might correlate to different clinical phenotypes of asthma and account for the different sensitivities and specificities of both types of challenges in assessing AHR.

In conclusion, our study shows that the mechanisms of PC₂₀ AMP and PC₂₀ methacholine differ in patients suffering from moderate-to-mild allergic asthma. Indeed, AMP challenge elicits a response in the lung periphery, including the furthest reaches of the terminal bronchioles zone (i.e., preacinar zone), whereas methacholine elicits a response in less peripheral airways. This suggests that while PC₂₀ AMP is able to detect bronchial responsiveness up to very peripheral airways, the reactivity reflected by PC₂₀ methacholine is restricted to less distal airways.

**APPENDIX A**

To be comparable to N₂ results reported in the literature, He and SF₆ concentrations may be normalized as follows:

\[
C_{\text{norm}} = \frac{C_{\text{N}_2} - C_{\text{act}}}{C_{\text{in}}}
\]

where \(C_{\text{norm}}, C_{\text{act}}, \) and \(C_{\text{in}}\) are the normalized, the actual, and the inspired concentrations, respectively. \(C_{\text{N}_2}\) is the N₂ concentration in dry air. \(C_{\text{in}}\) equals 5% and \(C_{\text{act}}\) equals 78.09%.

Given that the slope is the derivative of the concentration, the normalized \((S_{\text{norm}})\) and the actual \((S_{\text{act}})\) slopes are related by

\[
S_{\text{norm}} = -\frac{C_{\text{N}_2}}{C_{\text{in}}} S_{\text{act}} = -15.6 \cdot S_{\text{act}}
\]
APPENDIX B

The impact of proximal bronchoconstriction on the diffusion front of concentration during inspiration has been simulated using a model of inert gas transport (24). This model considers the simultaneous transport by axial molecular diffusion and convection (bulk flow) in geometrical boundaries based on the Weibel's symmetrical model (40).

Molecular diffusion coefficients equal to 0.6 and 0.1 cm²/s were considered for He and SF₆, respectively. An inspiration of 1 liter from a FRC of 3,700 ml was simulated with an inspiratory flow of 500 ml/s. Inspired concentrations were considered equal to 1.

Bronchoconstricted areas were simulated by dividing the considered airways cross sections by two, which is likely an overestimation of the bronchoconstriction due to a challenge (20% loss of FEV₁).

Figure 3A shows the diffusion fronts of He and SF₆ without bronchoconstriction. Figure 3B mimics bronchoconstriction up to a generation where transport is still purely convective for both gases (generation 14). Figure 3C simulates a bronchoconstriction up to He diffusion front (generation 17).

As shown on Fig. 3B, constriction arising where gas transport is purely convective does not affect the distal diffusion front. Moreover, when the constriction goes up to He diffusion front (Fig. 3C), its shape is marginally affected but not its position. Due to this insensitivity to bronchoconstriction, the diffusion fronts of both gases target stable sites of the bronchial tree. Hence pre- and postchallenge conditions may be compared, as may AMP and methacholine challenges.

GRANTS

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DISCLOSURES

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