Peripheral mononuclear leucocyte $\beta$ adrenoceptors and non-specific bronchial responsiveness to methacholine in young and elderly normal subjects and asthmatic patients

Martin J Connolly,* Joseph J Crowley, Christopher P Nielson, Nirmal B Charan, Robert E Vestal

Abstract

Background – As $\beta$ adrenoceptor dysfunction occurs in both the normal elderly subject and in young asthmatic patients, the hypothesis was examined that age related $\beta$ adrenoceptor changes are important in the pathogenesis of late onset asthma in old age.

Methods – Subjects were non-smokers who comprised 17 young normal subjects of mean (SE) age 29·4 (1·3) years, 17 elderly normal subjects of 67·2 (1·3) years, seven young asthmatic patients of 31·0 (2·8) years, and 17 elderly asthmatic patients of 68·5 (1·4) years. All asthmatic patients withheld inhalers for 12 hours and oral treatment for 24 hours before each study day. Subjects underwent an inhaled methacholine challenge (Newcastle dosimeter method) on two non-consecutive days. The slope of the flow at 50% of the vital capacity (FEF$_{50}$) dose-response curve was derived from the percentage fall in FEF$_{50}$ divided by methacholine dose (sFEF$_{50}$). Beta-adrenoceptor density (Bmax) and affinity ($\%$K$_H$) were determined with ($^{125}$I)iodocyanopindolol as the radioligand in membranes prepared from mononuclear leucocytes.

Results – Log sFEF$_{50}$ was shown to be reproducible (repeatability coefficient 0·41) on the two study days and was inversely related to $\%$K$_H$ but not to Bmax. Multiple regression analysis (all 58 subjects, overall $R^2$ = 0·57) revealed an inverse relation between log sFEF$_{50}$ and $\%$K$_H$, and between log sFEF$_{50}$ and Bmax. The inverse relation between log sFEF$_{50}$ and $\%$K$_H$ was preserved whereas that between log sFEF$_{50}$ and Bmax was lost when young asthmatic subjects or when all asthmatic subjects were excluded from multiple regression analysis.

Conclusions – The $\beta$ adrenoceptor dysfunction observed in late onset asthma may be similar to that seen during ageing. Thus late onset asthma may represent the extreme of a spectrum of age associated $\beta$ adrenoceptor dysfunction.

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Although most elderly asthmatic patients first develop asthma in adulthood, the pathogenesis of late onset asthma is poorly understood. Reduced $\beta$ adrenoceptor responsiveness is seen in young asthmatic patients and in normal elderly subjects. Increased non-specific bronchial responsiveness, determined by methacholine or histamine challenge, is characteristic of asthma, its degree correlating with the severity of the asthma. Beta-receptor dysfunction has long been postulated as a cause of increased non-specific bronchial responsiveness and of asthma itself. This should imply an increasing prevalence of high levels of non-specific bronchial responsiveness with increasing age, and a relation between the degree of $\beta$ adrenoceptor function and the level of non-specific bronchial responsiveness. However, evidence of the former is confusing and the latter hypothesis has been little investigated.

We have examined the hypothesis that age related $\beta$ adrenoceptor dysfunction occurs in late onset asthma by assessing: (1) mononuclear leucocyte membrane $\beta$ adrenoceptor density and affinity in elderly, late onset asthmatic and elderly non-asthmatic subjects, and in young asthmatic and non-asthmatic subjects; and (2) the relation between non-specific bronchial responsiveness to methacholine and the above in vitro indices of $\beta$ adrenoceptor function in all four subject groups.

Methods

Subjects

Healthy young (18–50 years) and elderly (60–85 years) non-asthmatic subjects, and young (22–42 years) and elderly (60–85 years) asthmatic subjects who were otherwise healthy were recruited from the community. Sixteen of the healthy young subjects had participated in a previous study using an identical protocol and their data are included for comparison with the other groups. In addition, data on the sensitivity of the neutrophil respiratory burst to inhibition by isoproterenol in many of the subjects have been reported previously.

Table 1 gives subject characteristics for each group. Asthmatic subjects were those with variable respiratory symptoms and documented 20% variability in forced expiratory volume in one second (FEV$_1$), either spontaneously or following a single dose of inhaled $\beta$ agonist. Non-asthmatic (normal) subjects had...
Beta-adrenoceptors and asthma in old age

Table 1  Mean (SE) characteristics of study subjects

<table>
<thead>
<tr>
<th>Subject group</th>
<th>n</th>
<th>Age (years)</th>
<th>Duration of baseline asthmatic (years)</th>
<th>Baseline FEV_1 (%predicted)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young normal</td>
<td>17</td>
<td>29-4 (1-3)</td>
<td>—</td>
<td>105-9 (2-6)</td>
</tr>
<tr>
<td>Young asthmatic</td>
<td>7</td>
<td>31-0 (2-8)</td>
<td>11-9 (5-1)</td>
<td>100-8 (3-7)</td>
</tr>
<tr>
<td>Elderly normal</td>
<td>17</td>
<td>67-2 (1-3)</td>
<td>—</td>
<td>109-1 (3-4)</td>
</tr>
<tr>
<td>Elderly asthmatic</td>
<td>17</td>
<td>68-5 (1-4)</td>
<td>18-8 (5-4)</td>
<td>80-9 (3-0)</td>
</tr>
</tbody>
</table>

* Determined according to standards published by Morris.58

no respiratory or atopic history and normal baseline lung function. Exclusion criteria for all groups included: cognitive impairment; pregnancy; cardiac disease; thyroid disorder; current cigarette smoking or smoking history of >10 pack years; respiratory infection, severe wheeze, medication change, or antihistamine treatment within six weeks; past or present treatment with β adrenergic antagonists, calcium antagonists or angiotensin converting enzyme (ACE) inhibitors; baseline FEV_1 (each study day) less than 60% of predicted.

Volunteers were screened by history, physical examination, and electrocardiography. A pregnancy test was performed where appropriate. Subjects refrained from drinks containing caffeine and oral medication for 24 hours and from inhalers for at least 12 hours before each study period (48 hours for long acting medication).

Of the 17 elderly asthmatic patients four were receiving no drugs and 13 were taking anti-asthma medication (some on multiple medication). Four subjects used occasional inhaled β agonists only (less than once daily), nine used regular inhaled β agonists, seven used methylxanthines, four inhaled steroids, and five received inhaled cromoglicate. Of the seven young asthmatic patients six used inhaled β agonists regularly and two regularly inhaled steroids.

STUDY PROTOCOL

Subjects were studied on non-consecutive days within a seven day period according to a protocol detailed elsewhere.29 Briefly, the protocol comprised the following:

Day 1: Subjects rested semirecumbent for 30 minutes, following which they sat up and inhaled methacholine was administered (initial dose 1-5 μg methacholine chloride) by a modified Newcastle dosimeter method.30 A single measurement of maximal expiratory flow at 50% of vital capacity (FEF_50) was obtained immediately before each dose. Alternative end points were 35% fall in FEF_50 or administration of the maximum cumulative dose of 6-4 mg methacholine. FEF_50 was monitored until it returned to normal. A bronchodilator was not given. The output of 12 Acorn “System 22” Turbo Nebulisers (Medic-Aid Ltd, Bognor Regis, West Sussex, UK) was maintained at 10 μl per nebulisation (standard deviation 0-9-2.5%) by computer controlled variation of nebulisation time.

Day 2: The protocol was identical to that for Day 1 except that a blood sample was obtained immediately before methacholine challenge.

Plasma was separated and frozen (−70°C) for catecholamine and IgE assays and mononuclear leukocytes were harvested from whole blood for assay of mononuclear leucocyte β adrenoceptor characteristics.

All subjects gave written informed consent to participate in the study which was approved by the Human Subjects Committee of the University of Washington and the Research and Development Committee of the Boise Department of Veterans Affairs Medical Center.

ASSAY METHODS

The assay methods are described in detail elsewhere.28 Plasma catecholamine concentrations were measured by the radioenzymatic method of Peuler and Johnson,32 and plasma IgE was determined by an amplified immunoradiometric assay. Mononuclear cells were isolated by density gradient centrifugation with Ficoll-Hypaque at 4°C,33 counted, and then lysed.34 Prepared membranes were stored dry at −70°C. The Bradford method was used for protein determination.34 Beta-adrenoceptor density and affinity in mononuclear leucocyte membranes were determined with ([125I]iodocyanopindolol (ICYP, 2200 Ci/mmol, NEN Research Products, Boston, Massachusetts, USA), as previously described.29 Receptor density (Bmax) and affinity for antagonist (KD) were determined from saturation isotherms by computer analysis using Lunden I (Lunden Software Inc, Cleveland, Ohio, USA).35 Agonist competition curves were analysed with a two site model and InPlot (GraphPad Software, San Diego, California, USA), which employs the Marquardt algorithm for non-linear regression.36

STATISTICAL ANALYSIS

The following information was derived for each subject: (1) simplified slope37 of FEF_50 (sFEF_50) dose–response curve to methacholine (in duplicate). This is a measure of non-specific bronchial responsiveness with units expressed as the percentage fall in FEF_50/mg methacholine. In this analysis slope is positively related to non-specific bronchial responsiveness; (2) total number of β adrenoceptors/mg cell membrane protein (Bmax); (3) dissociation constants for isotope and isoproterenol; and (4) percentage of receptors in high affinity state (%KH).

The following analyses were performed: (1) reproducibility of sFEF_50 by the method of Bland and Altman;38 (2) Student’s unpaired t tests comparing IgE and catecholamine levels between groups; (3) one way analysis of variance (ANOVA) to compare in vitro results between groups, allowing for sex differences; and (4) linear and multiple regression analysis employing sFEF_50 as the dependent variable. Unless otherwise stated, results are expressed as mean (SE). Statistical significance was defined at the 5% level.
Results

RESPONSE TO METHACHOLINE

Elderly asthmatic subjects had a lower baseline FEV₁ (% predicted) than young normal subjects (p < 0.001), elderly normal subjects (p < 0.001), and young asthmatic subjects (p < 0.001). To evaluate reproducibility of the response to methacholine, for each subject the ratio of the simplified slope of FEF₂₀₀ Day 1 (sFEF₂₀₀ Day 1) to sFEF₂₀₀ Day 2 on a log scale (equivalent to difference of the logs on a linear scale) was plotted against the geometric mean of the pair on a log scale (equivalent to mean log sFEF₂₀₀ on a linear scale). The ratios did not differ significantly from unity (fig 1). The geometric means of sFEF₂₀₀ on Days 1 and 2 were 237.8% fall/mg (95% confidence limits for mean 133.3 to 499.5% fall/mg) and 211.1% fall/mg (95% confidence limits for mean 100.6 to 442.8% fall/mg), respectively. The geometric mean of the ratios of sFEF₂₀₀ Day 1 to sFEF₂₀₀ Day 2 was 1.110 (95% confidence limits for mean 0.982 to 1.254). Coefficient of repeatability was 0.408. Thus, the 95% confidence limits for repeat sFEF₂₀₀ determination were 0.39 × initial sFEF₂₀₀ to 2.56 × initial sFEF₂₀₀.

The geometric mean of sFEF₂₀₀ Day 2 was 6.47% fall/mg (95% limits for mean 4.65 to 9.00% fall/mg) in young normal subjects, 115.6% fall/mg (95% limits 43.3 to 308.6% fall/mg; p < 0.0001) young normal subjects in elderly normal subjects, 844.9% fall/mg (95% limits 123.6 to 577.6% fall/mg; p < 0.0001) young normal subjects, and 8030.2% fall/mg (95% limits 4638 to 13 903% fall/mg; p < 0.0001) elderly normal subjects in elderly asthmatic patients. The range of sFEF₂₀₀ Day 2 was 1.72 to 23.5% fall/mg in young normal subjects, 1.15 to 2020% fall/mg in elderly normal subjects, 23.0 to 19 329% fall/mg in young asthmatic subjects, and 1380 to 46 272% fall/mg in elderly asthmatic patients.

Table 2 Mean (±S.E) plasma levels of IgE and catecholamines

<table>
<thead>
<tr>
<th>Subject group</th>
<th>IgE (units/ml)</th>
<th>Adrenaline (pmol/l)</th>
<th>Noradrenaline (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young normal</td>
<td>45.2 (16.5)</td>
<td>217 (37)</td>
<td>1.62 (0.12)</td>
</tr>
<tr>
<td>Young asthmatic</td>
<td>212.6 (140.2)</td>
<td>191 (56)</td>
<td>1.97 (0.37)</td>
</tr>
<tr>
<td>Elderly normal</td>
<td>53.1 (17.4)</td>
<td>220 (31)</td>
<td>2.22 (0.23)</td>
</tr>
<tr>
<td>Elderly asthmatic</td>
<td>100.0 (38.7)</td>
<td>258 (79)</td>
<td>2.65 (0.32)</td>
</tr>
</tbody>
</table>

Table 3 Characteristics of β adrenoceptors in membranes of peripheral mononuclear leukocytes

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Bmax (fmol/mg)</th>
<th>%Kᵣ</th>
<th>Kᵤ (nmol/l)</th>
<th>KDL (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young normal</td>
<td>45.57 (6.25)</td>
<td>60.68 (3.76)</td>
<td>218 (138-344)</td>
<td>4.79 (2.68-6.85)</td>
</tr>
<tr>
<td>Young asthmatic</td>
<td>26.63 (4.77)</td>
<td>49.21 (9.54)</td>
<td>134 (58-308)</td>
<td>3.92 (1.23-12.92)</td>
</tr>
<tr>
<td>Elderly normal</td>
<td>33.84 (4.31)</td>
<td>45.26 (5.83)</td>
<td>95 (47-192)</td>
<td>2.27 (1.31-3.95)</td>
</tr>
<tr>
<td>Elderly asthmatic</td>
<td>43.26 (5.65)</td>
<td>31.13 (4.30)</td>
<td>38 (17-85)</td>
<td>1.84 (1.14-2.96)</td>
</tr>
</tbody>
</table>

Data are mean (±S.E) except for KDL and KDL which are geometric means and for which the 95% confidence limits are shown in parentheses.

IgE and plasma catecholamines

As shown in table 2, elderly asthmatic patients had higher resting noradrenaline levels than young normal subjects (p = 0.005). ANOVA (sex and subject group as categorising variables) confirmed the difference in noradrenaline levels among all four groups (F₁,₃₂ = 4.21, p = 0.05), and between elderly asthmatic and young normal subjects (F₁,₃₂ = 7.76, p = 0.01), with similar trends between other groups except young normal v young asthmatic subjects, and elderly normal v young asthmatic subjects. Plasma levels of IgE and adrenaline did not differ significantly between groups.

Characteristics of β adrenoceptors

Since absolute values for dissociation constants of high (KDL) and low affinity (Kᵣ) receptors for isoproterenol were not normally distributed, these values were logarithmically transformed before analysis to ensure a normal distribution. As shown in table 3, for all four subject groups there was a significant intergroup difference in %Kᵣ (F₁,₃₂ = 6.07, p = 0.01), and in the dissociation constant (KDL) of the high affinity site (F₁,₃₂ = 5.18, p = 0.05), but no difference in Bmax or in the dissociation constant (KDL) of the low affinity site. Young normal subjects had significantly higher %Kᵣ values (F₁,₃₂ = 5.10, p < 0.05) and KDL values (F₁,₃₂ = 5.92, p < 0.05) than elderly normal subjects. Other variables were not significant. Although there was a trend towards a higher %Kᵣ in elderly normal subjects than in elderly asthmatic patients, the difference did not reach statistical significance. ANOVA comparing young normal with elderly asthmatic subjects showed differences for %Kᵣ (F₁,₃₂ = 25.9, p < 0.0001), KDL (F₁,₃₂ = 8.62, p < 0.01), and KDL (F₁,₃₂ = 15.46, p < 0.0005), but not for Bmax. Young asthmatic subjects had lower Bmax values than young normal subjects (F₁,₃₂ = 7.06, p < 0.02) or elderly asthmatic patients (F₁,₃₂ = 4.23, p < 0.05). Young asthmatic patients had higher %Kᵣ values than elderly asthmatic patients (F₁,₃₂ = 5.67, p < 0.05). KDL and KDL values did not differ between young asthmatic subjects and other groups. ANOVA revealed no sex differences in any analysis.

Although there was no relation between log sFEF₂₀₀ and Bmax, log sFEF₂₀₀ was inversely
Figure 2  Linear regression analysis of bronchial responsiveness (sFEF\textsubscript{50}, Day 2) v the percentage of \( \beta \) adrenoceptors in the high affinity state (%KH).

Since ANOVA comparing elderly normal and elderly asthmatic subjects had only demonstrated a trend towards an intergroup difference in %KH, a further multiple regression analysis was performed for these 34 subjects. It showed significant relations between log sFEF\textsubscript{50} and baseline FEV\textsubscript{1} (p < 0.005) and %KH (p < 0.05), but not between log sFEF\textsubscript{50} and Bmax.

No overall relation was shown between any in vitro variable and baseline lung function. For young or elderly asthmatic patients there was no relation between any in vitro variable and either duration of asthma or age of onset or between log sFEF\textsubscript{50} and asthma duration or age of onset.

### Discussion

This study has confirmed the reduction in mononuclear leucocyte membrane \( \beta \) adrenoceptor affinity with preservation of receptor density in normal elderly men and women. More importantly it has shown that, in comparison with young and elderly normal subjects, mononuclear leucocytes from elderly, late onset asthmatic patients have further reductions in affinity without a reduction in receptor density. Indeed, there was an inverse correlation between non-specific bronchial responsiveness (a marker of asthma severity) and receptor affinity, but not between non-specific bronchial responsiveness and receptor density.

These abnormalities in receptor affinity were not seen in young asthmatic patients, although in this latter group the previously described reductions in receptor density compared with young normal subjects were confirmed. Young asthmatic patients were also found to have lower receptor density than elderly asthmatic patients.

Multiple regression analysis employing an index of bronchial responsiveness as the dependent variable showed an inverse relation between responsiveness and both receptor affinity and receptor density when all subjects were included in the analysis. When young asthmatic subjects were excluded, however, the relation between responsiveness and density was lost but the inverse relation between responsiveness and affinity was preserved.

This was also true if only normal subjects were included in the analysis, strongly suggesting that the relation is a function of age (or ageing) rather than of asthma.

Taken together these results suggest that the mechanism or mechanisms responsible for \( \beta \) adrenoceptor dysfunction in late onset asthma are similar to those underlying age-associated \( \beta \) adrenoceptor dysfunction, and distinct from those associated with juvenile onset asthma. If true, then late onset asthma may represent the far end of a spectrum of age-associated \( \beta \) adrenoceptor dysfunction.

Although there is controversy as to the adequacy of the mononuclear leucocyte model in terms of the degree to which it reflects changes in airway \( \beta \) adrenoceptors, in vitro and in vivo abnormalities of \( \beta \) adrenoceptor function are well recognised in young asthmatics.\textsuperscript{28} and

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Table 4  Multiple regression analysis of non-specific bronchial responsiveness (log sFEF\textsubscript{50}, Day 2) against \( \beta \) adrenoceptor parameters, catecholamines, and IgE in all 58 subjects

<table>
<thead>
<tr>
<th></th>
<th>b</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>6.35</td>
<td>1.00</td>
<td>6.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>%KH</td>
<td>-0.013</td>
<td>0.006</td>
<td>-2.17</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Baseline FEV\textsubscript{1} (%)</td>
<td>-0.041</td>
<td>0.008</td>
<td>-5.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0.022</td>
<td>0.007</td>
<td>3.16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Bmax</td>
<td>1.125</td>
<td>0.056</td>
<td>20.2</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

b = partial regression coefficient; SE = standard error; t = Student’s t test; coefficient of multiple regression (R\textsuperscript{2}) adjusted for degrees of freedom = 0.57.

Table 5  Multiple regression analysis of non-specific bronchial responsiveness (log sFEF\textsubscript{50}, Day 2) against \( \beta \) adrenoceptor parameters, catecholamines, and IgE in young and elderly normal subjects only

<table>
<thead>
<tr>
<th></th>
<th>b</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.617</td>
<td>0.486</td>
<td>1.270</td>
<td>&gt;0.20</td>
</tr>
<tr>
<td>%KH</td>
<td>-0.012</td>
<td>0.006</td>
<td>-2.16</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Baseline FEV\textsubscript{1} (%)</td>
<td>0.030</td>
<td>0.006</td>
<td>4.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0.0008</td>
<td>0.0009</td>
<td>0.09</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IgE</td>
<td>-0.0008</td>
<td>0.0009</td>
<td>-0.09</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

b = partial regression coefficient; SE = standard error; t = Student’s t test; coefficient of multiple regression (R\textsuperscript{2}) adjusted for degrees of freedom = 0.56.

Table 6  Multiple regression analysis of non-specific bronchial responsiveness (log sFEF\textsubscript{50}, Day 2) against \( \beta \) adrenoceptor parameters, catecholamines, and IgE excluding young asthmatic subjects

<table>
<thead>
<tr>
<th></th>
<th>b</th>
<th>SE</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>3.76</td>
<td>0.98</td>
<td>3.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>%KH</td>
<td>-0.013</td>
<td>0.006</td>
<td>-2.34</td>
<td>0.02</td>
</tr>
<tr>
<td>Baseline FEV\textsubscript{1} (%)</td>
<td>-0.032</td>
<td>0.007</td>
<td>-4.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>-0.033</td>
<td>0.007</td>
<td>-5.06</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

b = partial regression coefficient; SE = standard error; t = Student’s t test; coefficient of multiple regression (R\textsuperscript{2}) adjusted for degrees of freedom = 0.74.

Bmax, KDH, KDL, catecholamines, and IgE were not significant.

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\( r = 0.51 \)
\( p < 0.001 \)
have been suggested as a cause of asthma. Similar in vitro and in vivo abnormalities have been described in normal elderly subjects and may be a consequence of the effects of ageing on the β-adrenoceptor-adenylyl cyclase system. Nevertheless, the limitations of the mononuclear leucocyte model must be acknowledged.

Lymphocytes and lung membranes of young asthmatic subjects have reduced numbers (density) of β-adrenoceptors. In contrast, normal elderly subjects have normal lymphocyte membrane receptor density but reduced receptor affinity for agonist. This is thought to represent functional uncoupling of the receptor from the adenylyl cyclase complex, and is associated with impaired receptor-mediated adenylyl cyclase activity. Impaired adenylyl cyclase activity is common to asthma and normal ageing. The possibility that late onset asthma represents the extreme end of a spectrum of ageing effects on the β-adrenoceptor pathway has not previously been examined, and in vitro studies on β receptors of elderly asthmatic subjects have not been reported.

It has been suggested that β-adrenoceptor dysfunction in ageing is due to downregulation by endogenous catecholamines. The higher resting noradrenaline levels in the elderly normal and elderly asthmatic subjects in this study are in general agreement with previous literature. Our failure to show a relation between noradrenaline levels and either receptor affinity or non-specific bronchial responsiveness level argues against the downregulation hypothesis.

The present finding of a greater degree of bronchial responsiveness in elderly normal than in young normal subjects is entirely consistent with the hypothesis of an association between late onset asthma (and therefore hyperresponsiveness) and age-associated β-adrenoceptor dysfunction. There is, however, disagreement in the literature as to the effect of age on non-specific bronchial responsiveness. Some studies have indeed suggested a higher level of responsiveness in the elderly while others have found no effect of age. In some of these latter cases, however, few or no subjects over 65 years were studied. Others examined the percentage of subjects with hyperresponsiveness (as opposed to absolute level of responsiveness) and at least one showed a trend towards hyperresponsiveness at the extreme end of the age range. A recent paper which again failed to show an age-associated increase in airway reactivity included only two of 85 subjects over 70 years. The young normal subjects in this same study displayed a surprisingly large range of non-specific bronchial responsiveness, measured as FEV₁ slope (over 400 fold, as opposed to 14 fold in the present study), and yet repeatability of their non-specific bronchial responsiveness measurement was not assessed. Two recent large studies report conflicting data on the effects of age on bronchial responsiveness in non-smokers.

There are three potential pitfalls with the novel hypothesis of the model of late onset asthma as an age-associated receptor-mediated disease. Firstly, we were unable to study large numbers of drug naive elderly asthmatic subjects. Thus, chronic treatment with β agonists may be implicated in downregulation of β-adrenoceptors. While this may partially explain our results, it would not explain why the receptor abnormality shown was exclusively one of reduced affinity, whereas similar studies on treated young asthmatics have shown reduced receptor density. Furthermore, it has been shown in older patients with chronic obstructive pulmonary disease that treatment with β agonists leads to a fall in density but not in agonist affinity in mononuclear leucocytes.

Secondly, the methacholine challenge itself (Day 1) may have affected in vitro β-adrenoceptor parameters (Day 2). Exposure of guinea pigs to high doses of inhaled acetylcholine for one week reduces β-adrenoceptor density in the lung membrane. However, affinity was unchanged and systemic β-adrenoceptors were not examined. Thus, even if as seems unlikely — systemic β-adrenoceptors were affected by one methacholine challenge two days before sampling, changes in density rather than affinity would be expected, with the greatest changes occurring in subjects receiving the largest methacholine dose (young normals). Such an effect would blunt rather than produce or exaggerate the differences seen in our study. The third and most important criticism of the suggestion that β-adrenoceptor ageing plays a major part in the pathogenesis of late onset asthma is quantitative rather than qualitative. Although acute intravenous β-blockade potentiates non-specific bronchial responsiveness in young non-asthmatic subjects, the potentiation is small (up to fourfold), whereas our elderly asthmatic patients possessed a degree of non-specific bronchial responsiveness 2000 times that of young normal subjects. However, this is likely to represent an oversimplification of the pathophysiology of chronic β-adrenoceptor dysfunction. The β-adrenergic system also modulates cholinergic neurotransmission at a prejunctional level with effects upon endogenous acetylcholine sensitivity up to 100 times more potent than its effects on response to exogenous cholinergic agents. Further, acute intravenous β-blockade would not produce immediate effects via other β-adrenergic-dependent mechanisms such as lung microvascular leakage, alveolar permeability, surfactant secretion, inhibition of mast cell mediator release and neutrophil lysozyme secretion, and stimulation of ciliated epithelial function. Modulation of any of these systems could be expected to result in changes in bronchial responsiveness. Indeed, late asthmatic responses, which are paralleled by increases in non-specific bronchial responsiveness, are associated with bronchial oedema and the release of prostaglandins, leukotrienes, and other mediators from mast cells and other sources. Furthermore, increases in epithelial permeability do enhance non-specific bron-
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Chronic responsiveness,34 and chronic asthma is associated with neutrophil accumulation in the airway and chronic oedema.55

We also showed the value of the methacholine challenge approach for the study of airway function in ageing. The methacholine challenge technique used for this study is well established.31 The use of FEF_{20} as the ventilatory parameter was dictated by the need to obtain measurable bronchconstriction in non-asthmatic subjects, and it is the small airway parameter which changes least with age.56 Expression of results as the simplified slope of the dose-response curve has been validated previously for FEV_{1}.37 The present study has shown its use with FEF_{20} to produce reproducible results (comparing favorably with more conventional indices using the same dosimeter in both young and elderly subjects).35

Our overall conclusion is that age-associated reduction in β-adrenoceptor affinity for agonist (in the absence of any reduction in β adrenoceptor density usually associated with juvenile onset asthma) plays a part in the aetiology of late onset asthma either by direct effects upon the β adrenoceptor system in the airway or, more possibly, by modulation of the response of airway inflammatory cells, mediator release, or other indirect mechanisms. Although it is likely that late onset asthma is an age-related phenomenon, additional studies will be necessary to fully elucidate the mechanisms involved.

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