Role of neutral endopeptidase in exercise-induced bronchoconstriction in asthmatic subjects

HEIDI W. F. M. DE GOUW, ZUZANA DIAMANT, EUGENIE A. P. KUIJPERS, JACOB K. SONT, AND PETER J. STERK

Department of Pulmonology and of Clinical Chemistry, Pharmacy, and Toxicology, Leiden University Hospital, 2300 RC Leiden, The Netherlands

De Gouw, Heidi W. F. M., Zuzana Diamant, Eugenie A. P. Kuijpers, Jacob K. Sont, and Peter J. Sterk. Role of neutral endopeptidase in exercise-induced bronchoconstriction in asthmatic subjects. J. Appl. Physiol. 81(2): 673–678, 1996.—The membrane-bound metalloproteinase, neutral endopeptidase (NEP), is a degrading enzyme of both bronchoconstrictor and bronchodilator peptides within the airways. To examine the role of NEP in exercise-induced bronchoconstriction (EIB) in asthmatic subjects, we used inhaled thiorphan, a NEP inhibitor, as pretreatment to a 6-min standardized exercise challenge. Thirty-six clinically stable asthmatic subjects participated in this double-blind, placebo-controlled, crossover study that was performed on 2 days separated by 48 h. Thiorphan was administered by two inhalations of 0.5 ml containing 1.25 mg/ml. Subsequently, exercise was performed on a bicycle ergometer at 40–50% of predicted maximal voluntary ventilation while inhaling dry air (20°C, relative humidity 6%). The airway response to exercise was measured by forced expiratory volume in 1 s (FEV₁) every 3 min, up to 30 min postexercise challenge, and was expressed both as the maximal percent fall in FEV₁ from baseline and as the area under the time-response curve (AUC) (0–30 min). The acute effects of both pretreatments on baseline FEV₁ were not different (P > 0.2), neither was there any difference in maximal percent fall in FEV₁ between thiorphan and placebo (P > 0.7). However, compared with placebo, thiorphan reduced the AUC by, on average, 28% [AUC (0–30 min, ±SE): 213.6 ± 47.7 (thiorphan) and 288.6 ± 46.0%fall·h (placebo); P = 0.047]. These data indicate that NEP inhibition by thiorphan reduces EIB during the recovery period. This suggests that bronchodilator NEP substrates, such as vasoactive intestinal polypeptide or atrial natriuretic peptide, modulate EIB in patients with asthma.

Asthma; membrane metalloendopeptidase; neuropeptides; natriuretic peptides, atrial; thiorphan.

NEUTRAL ENDOPEPTIDASE (NEP; EC 3.4.24.11) is a glycosylated zinc-dependent membrane-bound metalloproteinase with a broad selectivity, which preferentially cleaves peptides on the amino side of hydrophobic residues (29). In the lungs of different animal species and humans, NEP has been localized in the basal cells of the epithelium, alveolar type II cells, submucosal glands, airway smooth muscle, postcapillary venules, and nerves (18, 19).

NEP can be considered as a potential modulator of airway obstruction, which might be particularly relevant in diseases like asthma (23). Inhibition of NEP by inhaled thiorphan or phosphoramidon has been reported to be ineffective in changing baseline lung function or bronchial hyperresponsiveness to methacholine in both normal and asthmatic subjects in vivo (10, 11). Therefore, in mild and stable asthma, NEP substrates do not seem to be involved in the maintenance of airway patency. However, the potential role of NEP and its substrates during the development and recovery of acute airway obstruction, e.g., induced by a physiological challenge such as exercise, has not been elicited.

Standardized exercise challenges (31) are able to provoke bronchoconstriction in 70–80% of the asthmatic subjects, as reflected by a >15% fall in forced expiratory volume in 1 s (FEV₁). The major determinant for the severity of exercise-induced bronchoconstriction (EIB) is the level of ventilation during exercise (1). There is little doubt that water loss resulting in a transient hyperosmolarity and/or cooling of the airways is the physical stimulus for EIB, resulting in contraction of airway smooth muscle, vasodilation, and airway wall edema (1). As appears from in vitro and in vivo studies in animals and in humans, both cellular pathways and neurogenic mechanisms are likely to be involved in EIB. Apart from mast cell- and/or eosinophil-derived mediators (12, 22, 33), there is growing evidence that after hyperosmolar and/or cold dry air stimulation neurogenic activity may contribute to the subsequent airway obstruction (21, 28, 32, 34). These neural mechanisms particularly refer to noncholinergic nonadrenergic (nNANC) pathways (28, 32, 34), which can be either excitatory (eNANC), through NEP substrates such as substance P, neuropeptide Y, and calcitonin gene-related peptide, or inhibitory (iNANC) by, e.g., vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) (4, 5, 8). Finally, atrial natriuretic peptide (ANP) is another NEP substrate (3) potentially inhibiting EIB, since plasma immunoreactive ANP levels have been shown to rise between 3 and 15 min postexercise (30).

Hence, the role of NEP in EIB can be bifunctional. First, the observation of an increase of hyperpnea induced bronchoconstriction in guinea pigs (28) and of hypertonic saline-induced vascular permeability in the rat trachea (32) after pretreatment with phosphoramidon indirectly favors the involvement of the cNANC system in EIB, thereby suggesting a potentially protective role of NEP in EIB. Alternatively, any activation of the iNANC system and the rise in ANP levels (30) during exercise would imply an aggravating role of NEP in EIB.

In this study, we addressed the above hypotheses by examining the effect of pretreatment with the inhaled NEP inhibitor thiorphan on the development and recovery of EIB in patients with asthma. To that end, we investigated the maximal percent fall in FEV₁ and the area under the time-response curve (AUC) over a 30-min period following a standardized exercise test in
mildly-to-moderately asthmatic subjects in vivo after randomized pretreatment with inhaled thiorphan or placebo. We found that EIB was attenuated by inhaled thiorphan during the recovery phase between 15 and 30 min postexercise.

METHODS

Subjects. Thirteen nonsmoking atopic asthmatic volunteers, aged 18–31 yr (mean 24 yr), participated in the study. All had mild-to-moderate stable asthma, and symptoms were controlled by on-demand usage of inhaled short-acting β₂-agonists alone. Their atopic status was confirmed by a positive skin-prick test to at least one of the 16 common airborne allergen extracts (wheal ≥3 mm; Vivodiagnost, ALK, Benelux). Their baseline FEV₁ was ≥70% of predicted (27), and all were hyperresponsive to histamine (provocative concentration of histamine causing a 20% fall in FEV₁ < 4 mg/ml) (31). All subjects reported symptoms of EIB, which were confirmed by a fall in FEV₁ exceeding 15% from the baseline value after a 6-min exercise challenge at screening (Table 1). There was no history of upper respiratory tract infection or relevant allergen exposure during the 2 wk before the start of the study. The subjects had not used corticosteroids, antihistamines, sodium cromoglycate, nedocromil sodium, or theophyllines for at least 6 mo before the study. Before testing, they were asked to refrain from inhaled short-acting bronchodilators for at least 8 h and from caffeine-containing beverages for at least 4 h. The protocol was approved by the Medical Ethics Committee of the Leiden University Hospital, and all participants gave their written informed consent.

Study design. On two screening days before the study, the inclusion criteria were examined for each subject. The study had a placebo-controlled crossover design and was performed on 2 days with an interval of 48 h. On both study days, an exercise challenge was performed 10 min after double-blind randomized pretreatment with inhaled thiorphan or placebo. FEV₁ was measured every 3 min up to 30 min after cessation of the exercise challenge. Afterward, aerosolized salbutamol was administered (200 μg per metered-dose inhaler). The variability in baseline FEV₁ was not allowed to exceed 10% between both study days. Each subject attended the laboratory at the same time of day (22 h) on each study day.

Pretreatment. Thiorphan (DL-3-mercapto-2-benzyl-propanoylglycine; Sigma Chemical, St. Louis, MO) in normal saline, containing 1% human serum albumin (CLB, Amsterdam, The Netherlands) or placebo (normal saline containing 1% human serum albumin), was administered using a method that has previously been shown to be safe and effective in humans (10, 11). Thiorphan was stored at −20°C and warmed up on melting ice before nebulization. The aerosols were generated by an efficient and previously validated jet nebulizer (Malineckrodt Diagnostiek, Petten, The Netherlands) that is suitable for the administration of high doses of precious substances (10, 11). The dose of thiorphan was based on previous dose-finding experiments (11). The nebulizer was filled twice with 0.5 ml of the thiorphan solution (1.25 mg/ml), which was sprayed by compressed nitrogen during 1 min into a 30-liter collapsible drying chamber in which the droplets (saline mass median aerodynamic diameter 2.5 μm) rapidly evaporate to dry particles (mass median aerodynamic diameter 0.6 μm) (6). Subsequently, the aerosols, with a deposition fraction of ~50% within the intrapulmonary airways (6), were inhaled through a three-way valve box and a mouthpiece by tidal breathing for 3–4 min each, with the nose clipped. Oxygen was supplied through the mouthpiece at a flow of 4 l/min.

Exercise challenge. The exercise challenge was performed according to a standardized protocol (31) with some modifications, using a bicycle ergometer. The work intensity was selected for each individual subject to achieve a minute ventilation between 40 and 50% of his or her predicted maximal voluntary ventilation (35 × FEV₁ predicted) during 6 min. Compressed dry air (20°C, relative humidity 6% H₂O) was administered through an air-supply bag connected to a Hans Rudolph three-way valve. The subject wore a face mask covering the nose and the mouth together with a noseclip to ensure mouth breathing of dry air alone. The air was expired into the ambient air.

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Atopic Status*</th>
<th>FEV₁ %pred†</th>
<th>Histamine, mg/ml$</th>
<th>Postexercise FEV₁, %fall$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>26</td>
<td>192</td>
<td>5</td>
<td>110</td>
<td>3.27</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>22</td>
<td>181</td>
<td>3</td>
<td>87</td>
<td>1.54</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>26</td>
<td>174</td>
<td>7</td>
<td>90</td>
<td>0.61</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>25</td>
<td>175</td>
<td>3</td>
<td>85</td>
<td>0.84</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>18</td>
<td>169</td>
<td>3</td>
<td>87</td>
<td>1.15</td>
<td>22</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>25</td>
<td>188</td>
<td>1</td>
<td>87</td>
<td>2.83</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>31</td>
<td>179</td>
<td>6</td>
<td>73</td>
<td>0.14</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>26</td>
<td>176</td>
<td>5</td>
<td>80</td>
<td>2.35</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>22</td>
<td>173</td>
<td>5</td>
<td>91</td>
<td>1.71</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>18</td>
<td>178</td>
<td>3</td>
<td>82</td>
<td>0.44</td>
<td>27</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>24</td>
<td>164</td>
<td>4</td>
<td>86</td>
<td>0.28</td>
<td>30</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>25</td>
<td>180</td>
<td>4</td>
<td>81</td>
<td>0.49</td>
<td>32</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>24</td>
<td>173</td>
<td>4</td>
<td>100</td>
<td>0.65</td>
<td>33</td>
</tr>
</tbody>
</table>

FEV₁, forced expiratory volume in 1 s; * Atopic status determined by no. of wheal responses to 16 common airborne allergen extracts (Vivodiagnost, ALK, Benelux); † Baseline in predicted values in screening period; $ provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) in screening period; ‡ % fall in FEV₁ from baseline value after exercise challenge in screening period.
Table 2. Effect of either pretreatment on baseline FEV\textsubscript{1} before exercise challenge

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>After-before</th>
<th>$\Delta^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>3.80 ± 0.17</td>
<td>3.73 ± 0.15</td>
<td>0.054</td>
<td>0.24</td>
</tr>
<tr>
<td>Thiorphan</td>
<td>3.75 ± 0.19</td>
<td>3.72 ± 0.19</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

$^*$Difference in changes in baseline FEV\textsubscript{1} between the 2 pretreatments.

Methacholine control experiments. To exclude an effect of thiorphan on recovery from bronchoconstriction per se, placebo-controlled crossover control experiments were performed by using thiorphan or placebo as a pretreatment 10 min before methacholine challenge in eight asthmatic subjects who met the same inclusion requirements.

Methacholine challenge was performed according to a standardized procedure (31) by using methacholine (acetyl-β-methylcholine bromide) (Sigma Chemical) in normal saline. Serial doubling concentrations ranging from 0.3 to 40 mmol/ml in normal saline were aerosolized with the use of a nebulizer (model 646; DeVilbiss, Somerset, PA) operated by oxygen (output 0.13 ml/min) and connected to the central chamber of an inspiratory-expiratory valve box with an expiratory aerosol filter (Ultipor BB50; Pall, Portsmouth, UK). The aerosols were inhaled by tidal breathing for 2 min at 5-min intervals until FEV\textsubscript{1} fell by >10% from baseline value. The subjects wore a noseclip during aerosol inhalation.

Airway responses to methacholine were measured by FEV\textsubscript{1}. Before and immediately after thiorphan or placebo inhalation, three measurements of FEV\textsubscript{1} were recorded to calculate mean baseline FEV\textsubscript{1}. The mean value after pretreatment was used as the premethacholine baseline in the analysis. The recovery from the response to methacholine was measured every 3 min during 30 min following the challenge.

Analysis. The bronchoconstrictor response to exercise and methacholine were expressed as the maximal percent fall in FEV\textsubscript{1} from the baseline value and as the AUC over the 30-min period postchallenge (AUC\textsubscript{0-30 min}). Student's paired $t$-test was applied to test the differences in those variables between thiorphan and placebo. Subsequently, Student's paired $t$-test was applied at the individual time points to explore the differences between thiorphan and placebo pretreatment at each specific time point. $P$ values < 0.05 were considered statistically significant.

RESULTS

Safety. All subjects completed the study. Thiorphan was well tolerated in all subjects, and no reverse reactions occurred.

Exercise challenge. Baseline FEV\textsubscript{1} before pretreatment was not different between both randomized study days ($P > 0.3$) (Table 2). Similarly, there was no difference in the change in FEV\textsubscript{1} after thiorphan and placebo pretreatment ($P > 0.2$). Post-pretreatment FEV\textsubscript{1} was not different between the two pretreatments ($P > 0.6$).

The mean time-response curves after placebo and thiorphan are shown in Fig. 1. Exercise induced a maximal percent fall in FEV\textsubscript{1} from baseline of 17.6 ± 2.4 (SE)\% after thiorphan and 18.1 ± 2.1\% after placebo pretreatment, respectively. There was no significant difference in maximal percent fall in FEV\textsubscript{1} between both pretreatments ($P = 0.80$).

However, when analyzed as AUC for the 30-min period, thiorphan significantly attenuated the bronchoconstrictor response to exercise by an average 26% compared with placebo (mean AUC ± SE: 213.6 ± 47.7 and 288.6 ± 46.0\% fall·h, respectively; $P = 0.047$) (Fig. 2). On analyzing individual time points of the time-response curve, it appeared that this difference in AUC was predominantly due to the recovery period (15–30 min postexercise) (Fig. 1).

Methacholine control experiments. In the separate experiments using methacholine challenge, there was also no difference in changes in baseline FEV\textsubscript{1} after
thiorphan and placebo pretreatment ($P > 0.8$). Furthermore, no significant difference could be detected between thiorphan and placebo either in maximal percent fall in FEV$_1$ from baseline value (mean %fall in FEV$_1$ ± SE: 19.1 ± 4.5 for thiorphan and 20.6 ± 3.7 for placebo; $P = 0.6$) or in AUC$_{0.30	ext{ min}}$ (mean %fall·h ± SE: 225.8 ± 36.8 for thiorphan and 275.4 ± 53.0 for placebo; $P = 0.5$) (Fig. 3).

**DISCUSSION**

The results of the present study show that, in patients with asthma, inhibition of NEP by inhaled thiorphan significantly attenuates the bronchoconstrictor response to exercise when expressed as AUC over a 30-min period postchallenge. This does not occur after methacholine-induced bronchoconstriction. These findings suggest that bronchodilator peptides, which are sensitive to degradation by NEP, exert a protective effect during the recovery period of EIB and that this effect is not due to bronchoconstriction per se.

This is the first study examining the role of NEP and NEP substrates during EIB in asthmatic subjects in vivo. Previous animal experiments have addressed an analogous question by using hyperosmolar or cold/dry air as stimuli to the airways in vivo and in vitro. In guinea pigs, it has been reported that intravenously administered phosphoramidon, another NEP inhibitor, augmented the bronchoconstrictor response to isocapnic hyperpnea in vivo (28) and potentiated the increase of vascular permeability caused by hypertonic saline in vitro (32). Therefore, eNANC responses mediated by NEP substrates, such as substance P, NKA, and/or calcitonin gene-related peptide, seem to be involved in hypertonie saline- and hyperventilation-induced bronchoconstriction in guinea pigs. Because these physical stimuli are essentially those considered to be most prominent during exercise challenge, involvement of the eNANC system in human EIB might have been anticipated. Consequently, this would have implied a protective effect of NEP during EIB. Remarkably, however, our findings of the contrary argue against exercise-induced eNANC activity in asthma.

In the present study, we examined the role of NEP activity during exercise challenge by using inhaled thiorphan as a pretreatment. We believe that the observed effects of thiorphan can indeed be interpreted in terms of NEP inhibition, since the selectivity of thiorphan for NEP compared with, e.g., angiotensin-converting enzyme is 50-fold (29). In addition, it is unlikely that our findings can be explained by measurement errors, since the data have been obtained by means of validated methodology. First, the challenges were carried out in a standardized way (31), using dry air to increase the ventilatory stimulus to the airways. Second, we confirmed that inhalation of thiorphan per se failed to cause significant bronchodilation in normal as well as in asthmatic subjects in vivo (7, 10, 11). Cheung et al. (10) demonstrated that lung function does not change when comparing thiorphan and placebo up to 90 min postinhalation. Thus a direct bronchodilator effect cannot explain the improvement in FEV$_1$ between 15 and 30 min after exercise in the present study. Furthermore, we used the same dose of thiorphan as has been shown to potentiate the bronchoconstrictor response to isocapnic hyperpnea in vivo (28) and to inhaled metabisulfite in nonasthmatic subjects (7). Because potentiation of the latter challenge by thiorphan can be considered to be due to reduced breakdown of endogenous neuropeptides, it seems likely that the present dose of thiorphan has been sufficient to inhibit cleavage of NEP substrates released secondary to exercise challenge. To exclude an effect of thiorphan on recovery from bronchoconstriction per se, we performed control experiments using thiorphan as a pretreatment to methacholine challenge. We could not detect an effect of thiorphan on the recovery from methacholine-induced bronchoconstriction, thereby favoring a specific exercise-induced release of bronchodilator NEP substrates in asthma. Finally, to exclude possible differences in NEP activity based on atopy or environmental factors, we selected a homogeneous group of nonsmoking, atopic, mildly-to-moderately asthmatic subjects without a history of viral infection and not using steroid medication before and during the experiments.

There are at least two mechanisms that could possibly account for the protective effect of NEP inhibition by inhaled thiorphan on EIB: namely, iNANC responses (4, 8) and/or ANP (3, 25). iNANC responses are mediated by the release of neuropeptides such as VIP and NO, potentially resulting in relaxation of airway smooth muscle (4). In contrast to VIP, NO is not sensitive for degradation by NEP (29), suggesting that VIP might be responsible for the reduction of EIB by inhaled thiorphan. Although it has been reported that VIP-positive nerve fibers are absent in lung tissue of severe asthmatic patients at autopsy (24), Howarth et al. (15) showed no reduction of VIP-containing nerves.

![Fig. 3. Bronchoconstrictor response to methacholine challenge after pretreatment with either inhaled thiorphan or placebo, expressed as AUC$_{0.30\text{ min}}$ postmethacholine in 8 individual asthmatic subjects (mean AUC ± SE: 225.8 ± 36.8%fall·h for thiorphan and 275.4 ± 53.0%fall·h for placebo; $P = 0.5$).](image-url)
in bronchial biopsies of mildly asthmatic patients compared with nonasthmatic controls. Therefore, it cannot be excluded that diminished breakdown of VIP can be responsible for the attenuation of EIB in the subjects of the present study.

Apart from NEP, it has been shown that other enzymes, such as tryptase and chymase released from airway mast cells, are capable of rapidly degrading VIP (9). However, so far, this has not been confirmed in humans in vivo. Jarjour and Calhoun (17) could not demonstrate a change in histamine and tryptase levels in bronchoalveolar lavage fluid either shortly or 24 h after exercise challenge in patients with asthma. It can be argued that these assays may not have been sensitive enough to exclude the involvement of mast cell proteases in EIB. However, since it is unlikely that thiorphan affects tryptase or chymase enzyme activity, we believe that this metabolic pathway of VIP does not explain the present findings.

Inhibition of NEP by inhaled thiorphan might have had an indirect effect on NO synthesis, since interaction between VIP and NO has been described in smooth muscle of the gut (20). In several in vitro studies on human and animal gut smooth muscle cells, it has been shown that, apart from NO production in VIP-immunoreactive nerves, NO is mainly derived from muscle cells during nerve stimulation secondary to VIP activity (13). It can be speculated that similar VIP-induced activation of NO-dependent pathways may also occur in smooth muscle tissues in other organs (20), so that NEP inhibition by thiorphan in the present study may have increased NO production secondary to inhibiting VIP breakdown in the airways. Particularly, since in healthy subjects increased NO production during exercise has been detected (26), this hypothesis requires further investigation, e.g., by measuring NO synthesis in exhaled air in asthma.

ANP is another bronchodilator peptide that is degraded and inactivated by NEP (29). Inhaled thiorphan has been shown to enhance the ANP-induced attenuation of the bronchoconstrictor response to histamine (3). Apart from its predominantly atrial synthesis and release, ANP is also produced and stored in peripheral tissues such as the lung (14). Several biological activities of ANP in the airways, such as vasodilation, airway smooth muscle relaxation, and action on microvascular permeability have been reported (25). Indeed, infused ANP has been shown to produce bronchodilatation in asthmatic subjects in vivo (16). Recently, it has been demonstrated that oral administration of candoxatril, another NEP inhibitor, resulted in elevation of plasma endogenous ANP levels in stable asthmatic subjects, although an accompanying change in bronchomotor tone or bronchial responsiveness to histamine could not be detected (2). Interestingly, Rubinstein et al. (30) have shown that exercise, both in nonasthmatic and asthmatic subjects, is associated with a significant increase in plasma immunoreactive-ANP levels, which occurred between 3 and 15 min after cessation of exercise and returned to baseline values within 60 min. This time course of ANP release after exercise is in keeping with the predominant effects of thiorphan during the second part of the time-response curve in the present study. Therefore, our findings suggest that reduced breakdown of ANP may have contributed to the attenuation of EIB by thiorphan.

In conclusion, inhaled thiorphan specifically attenuates the bronchoconstrictor response to exercise in mildly-to-moderately asthmatic subjects during the recovery period between 15 and 30 min postexercise. Even though the effects of thiorphan in the present setup were relatively small, our observation suggests that bronchodilator NEP substrates modulate exercise-induced bronchoconstriction in asthma. Because KiH is a common problem in asthmatic patients, our observation may have clinical relevance. It implies that any potential dysfunction of NEP, e.g., secondary to respiratory virus infection, cigarette smoke, or occupational pollutants (23), would not be deleterious per se to mildly asthmatic subjects with exercise-induced symptoms. However, our findings should not be extrapolated to patients with severe asthma, in whom it cannot be ruled out that eNANC substrates of NEP predominate (19). Hence, development of NEP inhibitors for potential therapeutic intervention in asthma should await further clarification of the role of this enzyme and its substrates in the various categories of the disease.

Address for reprint requests: H. W. F. M. de Gouw, Lung Function Laboratory, C2-P, Dept. of Pulmonology, Leiden Univ. Hospital, PO Box 9600, NL-2300 RC Leiden, The Netherlands.

Received 28 July 1995; accepted in final form 14 March 1996.

REFERENCES


