Effect of Enalapril on Exhaled Nitric Oxide in Normotensive and Hypertensive Subjects

Hiroyuki Sumino, Tetsuya Nakamura, Tsugiyasu Kanda, Kunio Sato, Tetsuo Sakamaki, Takashi Takahashi, Yuichiro Saito, Jin Hoshino, Toshiaki Kurashina, Ryozo Nagai

Abstract—We investigated whether an angiotensin-converting enzyme (ACE) inhibitor increases the production of nitric oxide (NO) in exhaled air in normotensive and hypertensive subjects. In study 1, 8 normotensive male subjects received a single oral dose of enalapril (5 mg) or nitrendipine (10 mg) in a crossover manner. Exhaled air was collected at baseline, and at 2, 4, and 8 hours after administration of the drug. In study 2, 10 normotensive subjects (6 men and 4 women) and 10 hypertensive subjects (6 men and 4 women) received a single oral dose of enalapril (5 mg). Exhaled air was collected at baseline and at 2 and 4 hours after administration of the drug. In study 1, enalapril significantly increased the NO release rate from the lung in normotensive subjects (36.9±5.1 pmol/s at baseline versus 58.3±7.3 pmol/s at 4 hours, \( P<0.01 \)). Nitrendipine did not change the NO release rate. In study 2, enalapril significantly increased the release of NO from the lung in normotensive subjects (40.4±6.0 pmol/s at baseline versus 70.3±11.4 pmol/s at 4 hours, \( P<0.01 \)) but not in hypertensive subjects. ACE inhibition increased NO production from the lung in normotensive subjects but not in hypertensive patients. The reduction of angiotensin II production and/or the accumulation of bradykinin in the pulmonary tissue may be responsible for increased NO production in components of the lung, such as the pulmonary vascular endothelium, bronchial epithelial cells, macrophages, nasopharyngeal cells, and neurons. However, the effects of ACE inhibition on NO production from the lung differ between hypertensive subjects and normotensive subjects. (Hypertension. 2000;36:934-940.)

Key Words: angiotensin ■ bradykinin ■ hypertension, essential ■ nitric oxide

Endothelium-derived relaxing factor (EDRF) has been identified as nitric oxide (NO), a potent vasodilator. The attenuated endothelium-dependent vasodilation in patients with essential hypertension may be largely mediated by a decrease in the release or activity of NO. Decreased endothelial NO is considered to be either a cause or a consequence of hypertension.

NO is a highly unstable substance with a plasma half-life of only a few seconds; therefore, its direct measurement is difficult, particularly in vivo. Endogenously produced NO is assessed by measuring the serum levels of NOx, the products of NO metabolism, or by measuring endogenous NO present in expired air. The presence of endogenous NO in exhaled air has been demonstrated in humans. The cellular source of exhaled NO is not known, but it has been proposed that all NO synthase isoforms in the respiratory system contribute to NO release. Therefore, the amount of NO in expired air is an appropriate indicator of endogenously produced NO in the lung. We recently developed a method for measuring NO in exhaled air.

Angiotensin-converting enzyme (ACE) inhibitors improve impaired endothelium-dependent vascular relaxation in rats, but this effect of ACE inhibitors is not always demonstrated in humans. However, it has not yet been established whether oral administration of ACE inhibitors increase the NO production from the lung or the nitrite/nitrate (NOx) concentrations of systemic circulating venous blood in humans.

In this study, we investigated whether treatment with an ACE inhibitor would increase NO production in exhaled air in normotensive and hypertensive subjects. We measured the NO release rate from the lung before and after administration of enalapril or nitrendipine in normotensive subjects. We also examined the NO release rate from the lung before and after administration of enalapril in normotensive and hypertensive subjects.

Methods

Subjects
Normotensive subjects in both study 1 and study 2 underwent physical examinations to rule out the presence of disease. None of the subjects had evidence of active infection or respiratory disease. None was a smoker. None of the subjects had taken any prior medication. Subjects were also excluded if they had had any
The effect of enalapril on the NO release rate was examined in 10 hypertensive patients (6 men, 4 women). The effect of enalapril on the NO release rate was examined in 10 hypertensive patients (6 men, 4 women). All subjects received a single oral dose of enalapril (5 mg). After subjects rested for 30 minutes in the supine position in a quiet room, exhaled air and blood samples were collected, and blood pressure and heart rate were measured at baseline and at 2 and 4 hours after drug administration. The NO release rate was determined in exhaled air, and the serum level of NOx and plasma levels of bradykinin and renin activity were measured.

**Collection of Exhaled Air**

Samples of exhaled air were obtained and NO gas was analyzed and the rate of NO release determined as previously described. In brief, after application of a nose clip, air inspired through the mouth was exhaled through the mouth into a 6-L bag made of polyvinylfluoride film (Tedlar bag, Iuchi). A Teflon column (15 × 50 mm) was inserted between the mouthpiece and the Tedlar bag. The column was packed with 6.7 g of silica gel (particle size, 1.7 to 4.0 mm, Kanto Chemical). This column was used to dry the exhaled air sufficiently to prevent the condensation of vapor on the walls of the Tedlar bag. NO was not adsorbed by the silica gel and is stable in the Tedlar bag for several hours if the sample is dry and is not exposed to light. The study was performed only when the concentration of NO in the room air was <3 parts per billion (ppb). The room air concentration of NO was 1.2±0.6 ppb (0.6 to 2.8 ppb) immediately before each measurement.

**Analysis of NO and Determination of NO Release Rate in Exhaled Air**

The NO concentration was measured with a chemiluminescence analyzer (model GLN-32; Denki-Kagaku-Keiki). The flow rate for sampling was 800 mL/min. The limit of detection for NO was 0.2 ppb. The coefficient of variation was 2% at 20 ppb of NO.

Subjects were instructed to exhale air after making a full inspiratory effort at 6 flow rates that were determined arbitrarily by each subject. The flow rates ranged from very fast to very slow. Air volumes on the 6 efforts corresponded to each person’s vital capacity. Samples were collected in separate bags. The time from the start to the end of each sampling was measured with a stopwatch. Sample volumes were determined on a chart recorder (model 056; Hitachi).

The concentration of NO in exhaled air was positively correlated with the duration of the exhalation. The rate of NO release for each subject was calculated on the basis of the slope of a simple linear regression model of the relation between the concentration of gas and the duration of exhalation as follows: Release rate of NO (pmol/s) = slope (ppb/s) × average of 6 sample volumes (L) × 1/V(L) × 10^(-3), where V corresponds to the volume of 1 mole of dry air at ambient temperature (24.5 L at 25°C at 760 mm Hg). The release rate was constant (35±4 pmol/s) in 5 control subjects who repeated the protocol 10 times over a 2-week period. In addition, the NO release rate at 0.2, 4, and 8 hours (37±5 pmol/s, 36±5 pmol/s, 37±3 pmol/s, 36±4 pmol/s, respectively) was not changed for 8 hours in 5 control subjects.

**Measurement of Serum Level of NOx**

Samples of venous blood were centrifuged at 500g for 10 minutes at 4°C. The resulting serum samples were stored at −70°C until assayed. For NOx measurements, 0.125 mL of thawed serum was added to 0.5 mL of 75 mmol/L ZnSO₄ solution, to which 0.625 mL of 55 mmol/L NaOH was then added. The final pH was maintained between 7.0 and 7.5. Treated samples were mixed by vortexing, allowed to stand for 10 minutes, and then centrifuged at 5000g for 10 minutes. The resulting supernatant was applied to a copperized cadmium reduction column to reduce nitrate to nitrite and subsequently reacted with the Griess reagent with the use of an autoanalyzer (TCI-NOX 1000; Tokyo Kasei Kogyo). The absorbance of the reaction mixture at 540 nm was measured by spectrophotometry. The limit of nitrite detection was 0.2 μmol/L.

**Measurement of Plasma Level of Bradykinin**

For measurement of bradykinin, samples were placed in siliconized vacuum tubes containing aprotinin, soybean trypsin inhibitor, protamine sulfate, and disodium EDTA. All tubes were placed in a box filled with ice until they were centrifuged at 500g for 10 minutes at

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**TABLE 1. Subject Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study 1 Normotensive</th>
<th>Study 2 Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
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<td>10</td>
<td>10</td>
</tr>
<tr>
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<td>6/4</td>
<td>6/4</td>
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<tr>
<td>Age, y</td>
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<td>44±3</td>
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<td>Body height, mm</td>
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<td>1630±12</td>
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<tr>
<td>Body wt, kg</td>
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<td>63±3</td>
<td>65±4</td>
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<td>%VC</td>
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<td>93±4</td>
<td>92±2</td>
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<tr>
<td>FEV₁%</td>
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<td>84±3</td>
<td>88±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
TABLE 2. Changes in Hemodynamics in 8 Normotensive Subjects Treated With ACE Inhibitor or Calcium Antagonist (Study 1)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACE Inhibitor (n=8)</th>
<th>Calcium Antagonist (n=8)</th>
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<tbody>
<tr>
<td>SBP, mm Hg</td>
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<td></td>
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<tr>
<td>Baseline</td>
<td>122±3</td>
<td>123±2</td>
</tr>
<tr>
<td>2 h</td>
<td>116±1</td>
<td>118±4</td>
</tr>
<tr>
<td>4 h</td>
<td>119±4</td>
<td>115±4</td>
</tr>
<tr>
<td>8 h</td>
<td>119±2</td>
<td>115±2</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>76±3</td>
<td>78±3</td>
</tr>
<tr>
<td>2 h</td>
<td>73±2</td>
<td>76±3</td>
</tr>
<tr>
<td>4 h</td>
<td>72±3</td>
<td>73±2</td>
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<tr>
<td>8 h</td>
<td>75±1</td>
<td>75±4</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>64±2</td>
<td>66±3</td>
</tr>
<tr>
<td>2 h</td>
<td>69±2</td>
<td>75±5*</td>
</tr>
<tr>
<td>4 h</td>
<td>73±2*</td>
<td>71±5</td>
</tr>
<tr>
<td>8 h</td>
<td>68±2</td>
<td>71±4</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

Values are mean±SEM.

*p<0.05 vs baseline, ANOVA.

Measurement of Plasma Level of Renin Activity

Venous blood samples from the contralateral arm were collected in tubes containing EDTA-2Na (1 mg/mL) and promptly chilled in an ice bath. After samples of venous blood were centrifuged at 500g for 10 minutes at 4°C and plasma was removed, aliquots of the plasma sample were stored at −80°C until analysis. The plasma bradykinin level was measured by radioimmunoassay.10

4°C. Supernatants were stored in sealed polypropylene tubes at −80°C until analysis. The plasma bradykinin level was measured by radioimmunoassay.10

**Measurement of Plasma Level of Renin Activity**

Venous blood samples from the contralateral arm were collected in tubes containing EDTA-2Na (1 mg/mL) and promptly chilled in an ice bath. After samples of venous blood were centrifuged at 500g for 10 minutes at 4°C and plasma was removed, aliquots of the plasma sample were stored at −20°C until use. Plasma renin activity (PRA) was determined by radioimmunoassay with radioimmunoassay kits obtained from SRL Radioisotope Laboratories, Tachikawa.

**Drugs**

Enalapril and nitrendipine were provided by Banyu Pharmaceutical Co and Yoshitomi Pharmaceutical Co, respectively.

**Statistical Analysis**

Two-way ANOVA was used to compare measurements of blood pressure, the NO release rate, the heart rate, the serum level of NOx, and the plasma levels of bradykinin and renin activity at baseline and 2, 4, and 8 hours after administration of enalapril or nitrendipine in normotensive subjects in study 1 and at baseline and 2 and 4 hours after administration of enalapril between normotensive and hypertensive subjects in study 2. When ANOVA yielded a significant result, the location of difference was determined by the unpaired or paired t test. All values are expressed as mean±SEM. A value of P<0.05 was accepted as statistically significant.

**Results**

**Study 1**

Before treatment, there were no differences in blood pressure, heart rate, PRA, plasma bradykinin, and the serum NOx concentration between subjects treated with the ACE inhibitor or the calcium antagonist (Tables 2 and 3). Enalapril significantly increased heart rate (P<0.05) and PRA (P<0.01) at 4 hours compared with baseline levels. Blood pressure, plasma bradykinin, and serum NOx showed no significant changes 8 hours after drug administration. Nitrendipine significantly increased heart rate at 2 hours compared with the baseline level (P<0.05). Blood pressure, PRA, bradykinin, and serum NOx showed no significant changes 8 hours after administration of nitrendipine.

In enalapril-treated subjects, the NO release rate from the lung was significantly higher at 4 hours (58.3±7.3 pmol/s) than at baseline (36.9±5.1 pmol/s) (P<0.01) (Figure 1). The NO release rate from the lung was not significantly changed 8 hours after administration of nitrendipine (Figure 1).

![Figure 1. Time course of NO release from lung before and after administration of enalapril or nitrendipine in normotensive subjects (n=8). NO release rate from lung at 4 hours was significantly increased by enalapril administration (•, n=8) (P<0.01) but was not changed by nitrendipine (○, n=8).](image-url)
TABLE 4. Changes in Hemodynamics in Normotensive and Hypertensive Subjects Treated With Enalapril

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normotensive (n=10)</th>
<th>Hypertensive (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>119±4</td>
<td>152±5†</td>
</tr>
<tr>
<td>2 h</td>
<td>111±4*</td>
<td>143±4</td>
</tr>
<tr>
<td>4 h</td>
<td>107±6†</td>
<td>137±5†</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>80±1</td>
<td>103±3‡</td>
</tr>
<tr>
<td>2 h</td>
<td>72±3</td>
<td>95±3*</td>
</tr>
<tr>
<td>4 h</td>
<td>69±5*</td>
<td>88±4†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>66±2</td>
<td>68±2</td>
</tr>
<tr>
<td>2 h</td>
<td>68±2</td>
<td>72±3</td>
</tr>
<tr>
<td>4 h</td>
<td>70±4</td>
<td>77±3</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

Values are mean±SEM.

*P<0.05, †P<0.01 vs baseline, ANOVA.

‡P<0.001, hypertensive baseline vs normotensive subjects, unpaired t-test.

Study 2

Before treatment, both systolic (P<0.001) and diastolic (P<0.001) blood pressures were significantly higher in hypertensive subjects than in normotensive subjects (Table 4). There were no differences in heart rate, PRA, plasma bradykinin, and the serum NOx concentration between hypertensive and normotensive subjects before treatment (Tables 4 and 5). In normotensive subjects, the systolic blood pressure was significantly decreased at 2 hours (P<0.05) and at 4 hours (P<0.01) and diastolic blood pressure was significantly decreased at 4 hours (P<0.05) compared with the baseline level. The PRA was significantly increased at 4 hours compared with the baseline level (P<0.01). There were no changes in diastolic blood pressure, plasma bradykinin, and serum NOx 8 hours after administration of enalapril. In hypertensive subjects, systolic blood pressure was significantly decreased at 4 hours (P<0.01) and diastolic blood pressure was significantly decreased at 2 hours (P<0.05) and at 4 hours (P<0.01) compared with the baseline level, but PRA was significantly increased at 4 hours compared with the baseline level (P<0.01). Heart rate, plasma bradykinin, and serum NOx were not significantly changed 8 hours after administration of enalapril.

Before treatment, the NO release rate from the lung in hypertensive subjects was significantly lower than in normotensive subjects (P<0.05). The NO release rate from the lung was significantly increased in normotensive subjects at 4 hours (70.3±11.4 pmol/s) compared with baseline (40.4±6.0 pmol/s) (P<0.01) (Figure 2). The NO release rate from the lung was not significantly changed 4 hours after treatment in hypertensive subjects (Figure 2). The NO release rate was significantly higher in normotensive subjects than in hypertensive subjects at 2 and 4 hours (P<0.01 and P<0.01, respectively).

Discussion

The ACE inhibitor enalapril increased NO production from the lung in normotensive subjects. The calcium antagonist nitrendipine did not alter NO production from the lung. Before treatment, NO production from the lung in hypertensive subjects was lower than in normotensive subjects. Enalapril did not increase NO production from the lung in hypertensive subjects.

NO is present in exhaled air, at a stable concentration at rest. The level of NO during exhalation differs in various...
pathophysiological conditions: It is increased in patients with asthma,11 primary lung cancer,12 and systemic lupus erythematosus.13 Exhaled NO levels have also been found to increase with exercise14 and in response to orally administered l-arginine15 and intravenously infused nitroglycerin.16 Conversely, the exhaled NO level is decreased in patients with heart failure,17 hypertension,18 and acute respiratory distress syndrome.19 The exhaled NO level is also reduced by smoking.18 These findings suggest that the level of exhaled NO represents not only its regional production in the airway but also its systemic activity in various physiological or pathological conditions, suggesting that the amount of NO in expired air is a reliable indicator of in vivo NO production.

Schilling et al18 showed that the production of endogenous NO in the lung was impaired in patients with hypertension. Moreover, Forte et al20 reported that whole-body NO production in patients with essential hypertension was diminished under basal condition. Similarly, in the present study, the NO production from the lung in hypertensive subjects was significantly lower than normotensive subjects before treatment. Haefeli et al21 have demonstrated that quinaprilat induces arterial vasodilation mediated by NO in humans. Goldschmidt and Tallarida22 demonstrated that captopril evoked endothelium-dependent relaxations in rabbit aortic rings. Momboulì and Vanhoutte23 have suggested that endothelium-dependent relaxation induced by ACE inhibitors in perfused canine coronary arteries is mediated by an increase in local kinins. Although these previous studies assessed endothelium-dependent vasorelaxant actions, we measured exhaled NO in the present study. We found that an ACE inhibitor increased NO production from the lung in normotensive subjects. Hirooka et al24 have suggested that captopril may acutely improve impaired endothelium-dependent forearm vasodilation independent of a reduction in blood pressure per se in hypertensive patients. In the present study, however, enalapril did not increase NO production from the lung in hypertensive subjects. Although Hirooka et al24 demonstrated an increase in endothelium-dependent vascular relaxation, they did not directly measure NO production. Wiemer et al25 reported that the relative increase of carotid endothelial constitutive NO synthase expression induced by treatment with low doses of ramipril for ≈20 months was markedly higher for Wistar-Kyoto rats than for spontaneously hypertensive rats. They speculate that arrangement of NO synthase may differ between Wistar-Kyoto rats and spontaneously hypertensive rats.

In blood vessels, the converting enzyme is located mainly on the plasma membrane of endothelial cells facing the lumen.25,26 Thus, the endothelium is the principal target organ for ACE inhibitors. Recent studies have reported that the renin-angiotensin27 and kallikrein-kinin systems28 are also present in the vascular wall, especially in endothelial cells. Two possible mechanisms of ACE inhibition–induced increases in NO production have been proposed: (1) The decreased angiotensin II may increase NO production as the result of a decrease in superoxide production. Rajagopalan et al29 have demonstrated that angiotensin II–mediated hypertension in rats increases vascular superoxide production through membrane NADH/NADPH oxidase activation. (2) Bradykinin in the lung tissue increases the generation of NO through B2 receptor activation30,31 and the formation of prostacyclin.32

In the present study, enalapril significantly increased NO production from the lung in normotensive subjects. There was no change in the plasma level of bradykinin or NOx in normotensive or hypertensive subjects. ACE, a carboxy-terminal peptidyl dipeptidase, is more abundant on the luminal surface of pulmonary vascular endothelial cells33 than on other vascular beds. Because the measurement of exhaled NO is directly derived from the lung, it is possible that ACE inhibitors may cause a greater increase in NO release from the lung than in serum NOx in peripheral venous blood. It is possible that NO production in the systemic circulation is also increased by ACE inhibition but that the change is so small that NOx is rapidly excreted in urine without causing changes in the serum level of NOx. It is therefore more advantageous to use the amount of NO measured in expired air as an indicator of the endogenously produced NO levels than the circulating serum level of NOx. Thus, it is likely that increased bioavailability of NO induced by ACE inhibition may result in an increase in NO radicals in exhaled air but not in an increase in NO metabolites, measured as NOx in serum.

In the present study, PRA showed a 4-fold increase at 4 hours, confirming that enalapril exerts its actions in normotensive men. ACE inhibitors may increase NO production through accumulation of bradykinin. Given et al34 and Johnston et al35 reported that in normotensive and hypertensive subjects, a single 10-mg dose of enalapril significantly decreased plasma angiotensin II, inhibited plasma ACE activity, and increased PRA at 4 hours, but there were no significant changes in circulating bradykinin during the study. In the present study, the plasma level of bradykinin was not increased in normotensive or hypertensive subjects. We suggest that enalapril could not change circulating plasma bradykinin levels in normotensive and/or hypertensive groups but that enalapril may increase local bradykinin levels in pulmonary tissue in those groups. Plasma bradykinin is quickly degraded by peptidases in plasma and other biological fluids; the half-life of bradykinin in plasma is reported to be ≈17 seconds.36 Therefore, ACE inhibitor–induced increase in bradykinin may persist longer in lung tissue and may stimulate more NO production in the lung than in the vasculature.

Exhaled NO may be derived from pulmonary vascular endothelial cells, nerves, alveolar macrophages, and nasopharynx or airway epithelial cells.11,37,38 Decreased angiotensin II may increase NO bioavailability released from these tissues because of the associated decrease in superoxide anions. In addition, it has been reported that bradykinin releases NO from the tracheal epithelium39 and the pulmonary vascular endothelium.40 It is also possible that bradykinin releases NO from other tissues, such as pulmonary nerves, alveolar macrophages, and nasopharynx and airway epithelial cells.

Studies on the relation between calcium channel blockers and EDRF or NO production have yielded some conflicting
results. Rubanyi et al\textsuperscript{41} have found that Bay K 8644, a potent calcium channel activator, and the calcium-ionophore A23187 enhance EDRF synthesis in endothelial cells from canine femoral arteries. Bennett et al\textsuperscript{42} have shown that amlodipine has no effect on endothelium-dependent relaxation in mesenteric resistance arteries from spontaneously hypertensive rats. In contrast, Gunther et al\textsuperscript{43} have suggested that vasorelaxation induced by nitrendipine in coronary, basilar, and tail isolated pig arteries may be mediated in part by an increase in the release of NO. These discrepancies may be related to differences in the species, the drugs administered, or the experimental procedure. In the present study, nitrendipine did not alter NO production from the lung or the plasma level of NOx.

In conclusion, enalapril increased NO production from the lung in normotensive subjects but not in hypertensive subjects. The ACE inhibition–induced increase in NO production from the lung may be mediated by inhibition of angiotensin II production and/or the accumulation of bradykinin in lung tissue, such as the pulmonary vascular endothelium, bronchial epithelial cells, macrophages, nosophygeal cells, and neurons in the lung. This approach may influence the design of future studies assessing this system in normal subjects and in patients with hypertension and hypertensive complications.

Acknowledgments

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References

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