Original Contributions

Amlodipine Increases Nitric Oxide Production in Exhaled Air During Exercise in Patients With Essential Hypertension


Background: Endothelial production of nitric oxide (NO) is attenuated in patients with essential hypertension. We investigated whether treatment with amlodipine increased exhaled NO output (V˙NO) at rest and during exercise in patients with essential hypertension.

Methods: We studied the effect of amlodipine in seven untreated hypertensive patients. Cardiopulmonary exercise testing and NO measurement of exhaled air were performed on these patients before and after 2 months of amlodipine treatment.

Results: Amlodipine decreased blood pressure (BP) both at rest and during exercise (at rest: 147.1 ± 6.4 [SEM]v133.6 ± 5.4/89.9 ± 4.4 mm Hg, P < .05; at peak exercise: 224.9 ± 8.0/113.1 ± 5.3 v207.0 ± 6.0/100.7 ± 5.0 mm Hg, P < .05) without affecting heart rate (at rest: 67.6 ± 3.9 v70.4 ± 4.5 beats/min, P = .33; peak exercise: 146.4 ± 7.4 v144.0 ± 7.2 beats/min, P = .49). Amlodipine did not affect minute ventilation (VE) at rest or during exercise. It did not alter anaerobic threshold, peak oxygen uptake (peak VO2), or peak workload. However, after amlodipine treatment, VNO was significantly greater both at rest (130.8 ± 19.4 v180.4 ± 24.8 nL/min, P < .05) and at peak exercise (380.0 ± 47.5 v582.6 ± 74.3 nL/min, P < .05).

Conclusions: Amlodipine increased NO production, at least in the pulmonary circulation, in patients with essential hypertension. In addition to its antihypertensive effect, the enhancement of NO production by amlodipine in the vasculature of other organs may contribute to its beneficial effects on the cardiovascular system. Am J Hypertens 2004;17:729–733 © 2004 American Journal of Hypertension, Ltd.

Key Words: Nitric oxide, amlodipine, hypertension, exercise, exhaled gas analysis.

Endothelial cells are known to secrete nitric oxide (NO), a vasoactive substance. Nitric oxide is synthesized from L-arginine by NO synthase (NOS), which converts L-arginine to NO and L-citrulline. Nitric oxide is an important modulator of endothelial function and has protective effects on the cardiovascular system due to its antithrombotic, antiatherosclerotic, and anti-inflammatory actions.

In patients with essential hypertension, endothelial dysfunction is characterized by decreased activity of NO. Dihydropyridine calcium channel blockers are widely used in the treatment of hypertension and ischemic heart disease. In vitro studies have shown that dihydropyridine-type calcium channel blockers including amlodipine enhance NO production in the cardiovascular system. The augmented NO bioavailability may at least partially lead to improved endothelial function in patients with essential hypertension.7

We previously reported that NO in exhaled air can be used as an indicator of NO production in humans and that it increases during exercise due to increases in pulmonary blood flow.8 Under pathologic conditions, NO production varies. In patients with chronic heart failure, the increase in NO output during exercise is smaller compared to that observed in normal controls, leading to a blunted vasodilation.9 Enhanced NO production is also observed in patients with uncompensated liver cirrhosis, which is associated with systemic circulatory disturbances,10 and in patients with chronic renal failure or chronic glomerulonephritis who have increased levels of plasma cytokines.11,12
It is unclear whether amlodipine increase NO production in humans. Because NO is rapidly scavenged by hemoglobin, we quantified exhaled NO as an indicator of NO production in the lungs of patients with essential hypertension before and after amlodipine treatment. The NO measurement and exercise tolerance were simultaneously evaluated.

**METHODS**

**Subjects**

Seven untreated male patients with essential hypertension were enrolled for this study. Hypertension was defined as a systolic blood pressure (BP) at rest of >140 mm Hg or as a diastolic BP of >90 mm Hg. They were diagnosed by routine clinical examinations and patients with any cardiac, respiratory (including bronchial asthma), or other kind of infectious disease were excluded. Informed consent was obtained from each patient after the ethics committee of our institution approved the study protocol.

**Administration of Amlodipine**

After baseline symptom-limited exercise test and a breath-by-breath gas analysis, the patients were medicated with amlodipine besylate orally at a dose of 10 mg once a day for 2 months. Before and after the administration, exercise tolerance and exhaled NO was quantified according to the following protocol.

**Exercise Protocol and Expired Gas Analysis**

Patients performed a symptom-limited exercise on an electromagnetically braked upright cycle ergometer (Lood WLP-400 with ramp slope controller, Groningen, The Netherlands) at least 2 h after lunch. After a 4-min rest on the cycle ergometer, a 4-min warm-up exercise was started at 20 W and then the work rate was increased by 1 W every 6 sec. Patients were monitored by 12-lead electrocardiography using a stress system (ML-5000; Fukuda Denshi, Tokyo, Japan). Blood pressure was measured using an automatic indirect cuff manometer (STBP-780; Colin, Aichi, Japan) every minute. Expired gases were measured continuously in all patients on a breath-by-breath basis using an expired gas analyzer (RM-300; Minato Ikagaku Co., Osaka, Japan). Ventilatory parameters, including oxygen uptake (VO₂) and minute ventilation (VE), were calculated. The anaerobic threshold was determined mainly by the V-slope method.

**Measurement of Exhaled Nitric Oxide**

Nitric oxide concentration was continuously measured throughout the study, that is, at rest and during exercise. The NO concentration in exhaled air was determined in samples obtained with the patient during the exercise test. Each patient was instructed to inhale synthetic air (Taiyo-cho, Osaka, Thailand) free of NO (<3 ppb) through a mouthpiece, a hot wire flow meter, and a T valve. Exhaled air was continuously withdrawn from the tube with a vacuum pump at a rate of 200 mL/min and was introduced into a chemiluminescent analyzer (NOA; Sievers Instrumentals Inc., Boulder, CO). Measurement of NO concentration was based on the reaction of NO with ozone. The sensitivity of this analyzer ranged from 2 to 1000 ppb of NO, and the 90% response time was about 200 msec. The expired airflow was measured with the hot wire flow meter of the expired gas analyzer connected distal to the mouthpiece. The NO concentration and flow were continuously recorded with a data recorder (RD-120TE; TEAC Co., Tokyo, Japan) and were simultaneously analyzed by a computer. Those signals were also fed into the computer of another expired gas analyzer and exhaled NO output (VNO) was calculated every 10 msec according to the formula: NO output = NOex × volume of airflow, where NOex was the NO concentration in exhaled air.

**Statistical Analysis**

Values are expressed as mean ± SEM. Differences in anaerobic threshold, peak VO₂, and peak workload were compared using the Student paired t test.

Analysis of continuous variables before and after treatment with amlodipine was examined by two-way ANOVA of repeated measures. A t test with Bonferroni’s correction was used to evaluate differences between individual means. Stat View software (Version 5.0; SAS Institute Inc., Cary, NC) was used for statistical analysis. Statistical significance was defined as a probability rate of < .05.

**Results**

**Patient Characteristics**

Seven male patients were enrolled and all of them completed this study (Table 1). They were diagnosed with essential hypertension and were free from any cardiac or infectious diseases by routine clinical examinations.

**Changes in BP and Heart Rate**

All the patients enrolled in our study underwent cardiopulmonary examination and NO measurement before and 2 months after they were started on amlodipine. Amlodipine significantly decreased both systolic and diastolic pressures at rest and at every workload level (at rest: 147.7 ± 6.4/89.9 ± 4.4 vs. 133.6 ± 5.4/82.7 ± 3.9 mm Hg, P < .05; at peak exercise: 224.9 ± 8.0/113.1 ± 5.3 vs. 207.0 ± 6.0/100.7 ± 5.0 mm Hg, P < .05) (Fig. 1). Heart rate increased with the incremental of workload. Heart rate was not changed by amlodipine administration at each stage of workload statistically (rest: 67.6 ± 3.9 vs. 70.4 ± 4.5/min, P = .33; peak exercise: 146.4 ± 7.4 vs. 144.0 ± 7.2/min, P = .49) (Fig. 2).
Table 1. Characteristics of the patients enrolled in the study

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Mean ± SEM</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>49.7 ± 9.5</td>
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<tr>
<td>Sex (male/female)</td>
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</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>145.0 ± 4.4</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
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<tr>
<td>Risk factors</td>
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<td>Hypertension</td>
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<tr>
<td>Diabetes mellitus</td>
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</tr>
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<td>Smoking</td>
<td>2</td>
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<tr>
<td>Serum biochemistry</td>
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<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>102 ± 4</td>
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<tr>
<td>Serum total cholesterol (mg/dL)</td>
<td>203 ± 13</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dL)</td>
<td>169 ± 43</td>
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<tr>
<td>Serum blood urea nitrogen (mg/dL)</td>
<td>15.3 ± 2.7</td>
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<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>Uric acid (mg/mL)</td>
<td>5.5 ± 0.4</td>
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<tr>
<td>Brain natriuretic peptide (pg/mL)</td>
<td>15.6 ± 5.7</td>
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Values expressed as mean ± SEM.

Cardiopulmonary Exercise and Nitric Oxide Measurement of Exhaled Air

Peak VO₂, anaerobic threshold, and peak workload were almost within normal range before the administration of amlodipine. After amlodipine treatment, cardiopulmonary parameters (peak VO₂, anaerobic threshold, and peak workload) remained unchanged (Table 2). After the start of exercise, VE rapidly increased until peak exercise. VE was not influenced by amlodipine at any stage of workload (rest: 11.1 ± 0.8 v 10.9 ± 1.2 L/min, *P = .76; peak exercise: 60.9 ± 4.8 v 65.6 ± 5.9 L/min, *P = .23) (Fig. 3). VNO was significantly greater after amlodipine treatment at each stage (at rest: 130.8 ± 19.4 v 180.4 ± 24.8 nL/min, *P < .05; at peak exercise: 380.0 ± 47.5 v 582.6 ± 74.3 nL/min, *P < .05) (Fig. 4).

Discussion

As we have previously reported in healthy subjects, NO output in exhaled air incrementally increased the workload in patients with hypertension, both before and after administering amlodipine. The new finding of this study was that amlodipine increased NO in the exhaled air at rest and during exercise. Exhaled NO output has been shown to be a marker of endogenous NO production. Because NO is synthesized in various cells, such as vascular endothelium, airway epithelium, neurons, and macrophages, several sources can be considered as the origin of exhaled NO. Nitric oxide in the exhaled air presumably reflects the NO that is mainly produced in the lungs and airways, including the nose and upper airways, but not in the whole body due to the following reasons: 1) NO is rapidly scavenged by hemoglobin, and 2) NO concentration in the exhaled air increases after intravenous administration of nitroglycerin, whereas it decreases after intravenous administration of NO synthase inhibitors.

Because we collected NO in the exhaled air through a mouthpiece in this study, NO contamination from the nose and upper airways can be neglected. An increase in cardiac output during exercise causes an increase in shear stress, which triggers NO production and vascular dilation. VNO is also increased in patients with severe liver cirrhosis who have decreased systemic vascular resistance and increased cardiac output (i.e., hyperdynamic circulation). These findings support the hypothesis that exhaled NO comes from the vasculature in the lungs, although both constitutive and inducible NO synthase (NOS) exist in the lung epithelial cells.

Amlodipine has been shown to enhance NO availability. Zhang and Hintze reported that amlodipine, but not nifedipine or diltiazem, increases NO production from canine coronary microvessels through a kinin-dependent mechanism. The increased release of NO is not due to the blocking action of L-type calcium channels, as vascular endothelial cells do not express these channels.
blocking activity, released NO, whereas the S- enantiomer, which has L-type channel blocking activity, does not release NO.21 This NO release by the R+ enantiomer is dependent on the production of bradykinin, because it is blocked by a bradykinin antagonist. In addition to the kinin-mediated mechanism, an antioxidative action has been reported. Nifedipine, another L-type calcium channel blocker, increased NO bioavailability without significantly altering the endothelial NOS mRNA and protein expression, suggesting antioxidative protection.5 Amlodipine and nifedipine indirectly upregulate superoxide dismutase expression, a molecule that scavenges reactive oxygen species in the endothelium.6 Thus, amlodipine enhances endothelial NO availability by enhanced NO production and by diminishing NO degradation through antioxidative properties.22

Using plethysmography, NO production was shown to be attenuated in patients with hypertension, and the antihypertensive drugs amlodipine and enalapril restored forearm arterial responsiveness.7 The augmented NO activity by amlodipine may contribute to the restoration of endothelial function as well as a BP-lowering effect. The incremental release of NO observed by amlodipine in this study might be partially dependent on a reduction in BP. However, all BP-lowering drugs do not always increase NO release from the lungs. Sumino et al23 showed that nitrendipine does not change the levels of exhaled NO in normotensive or hypertensive subjects, whereas enalapril increases NO production from the lungs in normotensive subjects, but not in hypertensive patients. These results may suggest that a BP-lowering effect per se does not predominantly contribute to increased NO production. The different properties of amlodipine and nitrendipine or to the differences in the condition of the measurements may be the reason why nitrendipine did not increase NO production. All NO samples in the nitrendipine study were measured within 8 h of administration, whereas in our study we measured NO production before and after 2 months of treatment. The NO measurement of patients receiving other types of antihypertensive agents would further clarify the increase in NO bioavailability by antihypertensive agents.

Exhaled NO, however, could be an indicator of inflammation in the airways.24 We must always be careful concerning the origin of NO, because under pathologic conditions, NO production may be altered. Nitric oxide is also produced by macrophages in the lung and NO in exhaled air may reflect eosinophilic inflammation in asthmatic patients and can be a marker of allergic airway inflammation. In our study, patients with respiratory diseases, including bronchial asthma and respiratory infection, were excluded; thus, the augmented NO production would indicate restoration of endothelial function.

Some previous clinical trials involving patients with hypertension suggested that calcium channel blockers increase the occurrence of ischemic heart disease.25 In our study, amlodipine decreased BP, but did not increase heart rate at rest or during exercise. A smaller increase in sympathetic activation and reflex tachycardia may lead to

Table 2. Data of cardiopulmonary exercise before and after treatment with amlodipine

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
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<tr>
<td>Peak VO₂ (mL/min/kg)</td>
<td>28.2 ± 1.2</td>
<td>28.0 ± 2.1</td>
<td>NS (P = .84)</td>
</tr>
<tr>
<td>AT (mL/min/kg)</td>
<td>15.7 ± 1.6</td>
<td>15.8 ± 1.5</td>
<td>NS (P = .88)</td>
</tr>
<tr>
<td>Peak workload (W)</td>
<td>145.2 ± 7.6</td>
<td>145.0 ± 8.0</td>
<td>NS (P = .83)</td>
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Peak VO₂ = peak oxygen uptake; AT = anaerobic threshold; NS = not significant

Values expressed as mean ± SEM.

FIG. 3. Minute ventilation (VE) before and after treatment with amlodipine. Values of VE at each workload level are shown. Error bars indicate mean ± SEM. VE did not vary significantly at each workload level.

FIG. 4. NO output (VNO) at rest and during exercise. Error bars indicate mean ± SEM. Data at rest, 20 W, 50 W, 100W, and peak exercise are shown. *P < .05 versus before treatment.
good cardiovascular prognosis. As to exercise tolerance, amlodipine did not alter the peak VO₂, anaerobic threshold, or peak workload, mainly because those values of exercise tolerance in these patients were within normal range before its administration. Similarly, other BP-lowering drugs do not alter or even reduce exercise tolerance in hypertensive patients who do not have coronary artery diseases or heart failure. Reduced NO production during exercise is observed in patients with heart failure who have reduced exercise tolerance. Furthermore, we have previously reported that NO inhalation improves pulmonary hypertension and gas exchange in patients with chronic heart failure, and that NO inhalation improves exercise capacity by reducing excessive ventilation. The increased release of NO by amlodipine might cause an improvement in exercise tolerance in patients with heart failure who have reduced NO production during exercise.

In conclusion, our data show that amlodipine increases NO production in patients with hypertension. Although the present study did not explore the mechanism for the improved bioavailability of NO caused by amlodipine, this property may have beneficial effects in hypertensive patients.

Acknowledgments
We thank Miwa Kurano for excellent technical assistance.

References