Influence of Nitric Oxide Synthase and Adrenergic Inhibition on Adenosine-Induced Myocardial Hyperemia

Niels H. Buus, MD, PhD; Morten Böttcher, MD, PhD; Flemming Hermansen, MD; Mikael Sander, MD, PhD; Torsten T. Nielsen, MD, DMSc; Michael J. Mulvany, PhD, DMSc

Background—Myocardial perfusion during adenosine-induced hyperemia is used both in clinical diagnosis of coronary heart disease and for scientific investigations of the myocardial microcirculation. The objective of this study was to clarify whether adenosine-induced hyperemia is dependent on endothelial NO production or is influenced by adrenergic mechanisms.

Methods and Results—In 12 healthy men, myocardial perfusion was measured with PET in 2 protocols performed in random order, each including 3 perfusion measurements. First, perfusion was measured at rest. Second, either saline or the NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 4 mg/kg) was infused, and perfusion during adenosine-induced hyperemia was determined. Last, in both protocols, the α-receptor blocker phentolamine was infused, and perfusion during adenosine-induced hyperemia was determined again. Resting perfusion was similar in the 2 protocols (0.69±0.14 and 0.66±0.18 mL·min⁻¹·g⁻¹). L-NAME increased mean arterial blood pressure by 12±7 mm Hg (P<0.01) and reduced heart rate by 16±7 bpm (P<0.01). Adenosine-induced hyperemia (1.90±0.33 mL·min⁻¹·g⁻¹) was attenuated by L-NAME (1.50±0.55 mL·min⁻¹·g⁻¹, P<0.01). The addition of phentolamine had no effect on the adenosine-induced hyperemia (2.10±0.34 mL·min⁻¹·g⁻¹, P=NS). In the presence of L-NAME, however, when the adenosine response was attenuated, phentolamine was able to increase hyperemic perfusion (2.05±0.44 mL·min⁻¹·g⁻¹, P<0.05).

Conclusions—Inhibition of endogenous NO synthesis attenuates myocardial perfusion during adenosine-induced hyperemia, indicating that coronary vasodilation by adenosine is partly endothelium dependent. α-Adrenergic blockade has no effect on adenosine-induced hyperemia unless NO synthesis is inhibited. (Circulation. 2001;104:2305-2310.)

Key Words: adenosine • nitric oxide • perfusion • receptors, adrenergic, alpha • vasodilation

Pharmacological vasodilation of the coronary microcirculation is widely used in noninvasive diagnosis of coronary heart disease with myocardial perfusion imaging. Such drugs as adenosine and dipyridamole have also become important tools in scientific studies of the coronary circulation. With either adenosine or dipyridamole, a reduced hyperemic response has been demonstrated in numerous diseases with involvement of the myocardial circulation, such as coronary atherosclerosis,1 hypertension,2 microvascular angina,3 hyperlipidemia,4 and diabetes.5 Structural abnormalities of the coronary resistance circulation have been suggested to explain the reduced vasodilatory capacity in such conditions as hypertension6 and cardiomyopathy.7 But functional disturbances of the vascular smooth muscles or endothelial cell layer could also influence the response to coronary vasodilators.8 To explore which functional disturbances may cause a decrease in myocardial perfusion, it is important to consider the complex interplay between local vasodilator mechanisms and systemic homeostasis underlying the responses to such pharmacological agents as adenosine.

Adenosine-induced vasodilation may be dependent on nitric oxide (NO) production. Thus, in isolated coronary vessels, adenosine-induced vasodilation is attenuated by NO synthase inhibition,9 and in vivo studies in pigs have reached a similar conclusion.9 In humans, forearm plethysmographic studies have reached divergent results regarding the role of NO in adenosine-induced vasodilation.10,11 The heterogeneity of results may be due to any effects of NO being counteracted by a widespread sympathetic activation caused by adenosine,12,13 which could be of particular importance in the coronary circulation because of the abundance of coronary α-receptors in coronary arterioles.8,14 Under normal physiological conditions, α-adrenergic vasoconstriction in the heart is suppressed by myogenic or metabolic factors. In situations with diminished production of such local vasodilator substances as NO or increased sympathetic nervous activity, however, α-adrenergic vasoconstriction may predominate and limit myocardial hyperemia.

The purpose of the present study was to clarify whether adenosine-induced hyperemia is dependent on endothelial
NO production and whether \(\alpha\)-adrenergic mechanisms influence adenosine-induced hyperemia during preserved and inhibited NO synthesis.

### Methods

#### Study Group

Twelve healthy men 23 to 27 years old were studied. All participants were without a history of cardiovascular disease and diabetes; all had a normal clinical examination and received no medication. Fasting cholesterol was 4.5±1.0 mmol/L, and glucose was 4.5±0.5 mmol/L. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. All participants signed an approved consent form before entering the study.

#### Myocardial Perfusion

Myocardial perfusion was quantified with a positron emission tomograph (model Exact HR 961, Siemens/CTI) with intravenous \([^{13}\text{N}]\text{ammonia}\) as perfusion tracer. A detailed description of how perfusion is quantified has been published.\(^{17}\) For each determination of perfusion, 740 MBq of \([^{13}\text{N}]\text{ammonia}\) was injected over 15 seconds. At the time of injection, acquisition of a dynamic sequence of images (12 frames of 10 seconds) was started to obtain time-activity curves from the blood pool and from the myocardium. After this sequence, a 900-second static frame was acquired to obtain high-resolution images, allowing correct assignment of the regions of interest (ROIs). In 3 midventricular slices of the static images, 3 ROIs were allocated within the boundaries of the left ventricular myocardium in each territory of the 3 coronary arteries. These ROIs were subsequently copied to the serially acquired dynamic images. This enabled us to obtain myocardial time-activity curves for \([^{13}\text{N}]\text{ammonia}\).\(^{18}\) The arterial input function was obtained from a small ROI placed in the center of the left ventricular blood pool of each of the 3 selected static images, and these were copied to the serially acquired images. Averages of activity values from each frame in the 3 input functions were used for perfusion calculations. The myocardial time-activity curves were corrected for physical decay of \([^{13}\text{N}]\text{ammonia}\) and the effect of partial volume by assuming a uniform left ventricular wall thickness to be 10 mm, yielding a recovery coefficient of 0.82. Between 2 successive scans with \([^{13}\text{N}]\text{ammonia}\), a 15-minute transmission scan was obtained to correct for photon attenuation.

Myocardial perfusion was calculated by fitting the corrected myocardial and blood pool time-activity curves to a 2-compartment model for \([^{13}\text{N}]\text{ammonia}\). This model corrects for spillover activity from the left ventricular blood pool to the left ventricular myocardium.\(^{17}\) Because the average perfusion values did not differ between the different vascular territories, an average value for the entire myocardium is reported.

#### Study Protocol

All participants were scanned on 2 different occasions, at least 1 week apart, with 2 different scan protocols performed in random order (Figure 1). Each protocol consisted of 3 separate scans. First, a resting scan was performed, then a second scan was performed during intravenous infusion of adenosine (140 \(\mu\)g · kg\(^{-1}\) · min\(^{-1}\) from 3 minutes before to 3 minutes after the injection of \([^{13}\text{N}]\text{ammonia}\)). Sixty minutes before the adenosine infusion, either saline in protocol 1 or N\(^\circ\)-nitro-L-arginine methyl ester (L-NAME, 133 \(\mu\)g · kg\(^{-1}\) · min\(^{-1}\) for 30 minutes, equal to a total of 4 mg/kg) in protocol 2, was infused. The third scan determined, in both protocols, the myocardial perfusion during adenosine-induced hyperemia immediately after administration of the \(\alpha\)-adrenergic receptor antagonist phentolamine (5 mg over 2 minutes followed by 5 mg over 10 minutes). After termination of protocol 2, the participants received an infusion of L-arginine (200 mg/kg over \(\approx\)15 minutes) for reversal of the L-NAME effect.\(^{18}\)

On both study days, blood pressure and heart rate were determined by automated sphygmomanometry and continuous 12-lead surveillance ECG before and during the pharmacological interventions. All subjects refrained from intake of caffeine-containing beverages and food for \(\approx\)24 hours before the PET scans. L-NAME was obtained from Clinalfa. Phentolamine was delivered by Novartis Healthcare, and adenosine and L-arginine were prepared at the Aarhus University Hospital Pharmacy.

#### Calculations and Statistical Analysis

Data are presented as mean±SD. Myocardial vascular resistance is calculated as mean arterial blood pressure (MAP; diastolic pressure+1/3×pulse pressure) divided by myocardial perfusion. Myocardial perfusion reserve is defined as adenosine-induced hyperemia divided by baseline perfusion, and coronary vascular resistance index is defined as vascular resistance during hyperemia divided by resistance at baseline. The rate-pressure product is defined as the product of systolic blood pressure and heart rate. The 1-way ANOVA for repeated measures or the paired \(t\) test was used for comparisons of the effect of saline and L-NAME on outcome measurements. Comparisons of \(>\)2 mean values were modified according to Bonferroni if demonstrated to be significant. Probability values of \(P<0.05\) were considered statistically significant.

#### Results

### Effects of L-NAME, Adenosine, and Phentolamine on Blood Pressure and Heart Rate

L-NAME induced an increase in MAP and a decrease in heart rate (Table). The time course of blood pressure increase and heart rate reduction is shown in Figure 2. After the L-NAME infusion, MAP increased by 12±7 mm Hg (Figure 2A). The increase in MAP was primarily due to an increase in diastolic blood pressure (14±6 mm Hg), whereas systolic blood pressure increased by only 5±7 mm Hg. Heart rate was reduced by 16±7 bpm after infusion of L-NAME (Figure 2B).

After saline treatment, adenosine alone increased heart rate by 21±13 bpm (Table), whereas adenosine in the presence of...
Effects of Adenosine on Myocardial Perfusion and Myocardial Vascular Resistance

Myocardial perfusion measurements and calculations of vascular resistance are shown in Figure 3. Resting perfusion (0.69±0.14 versus 0.66±0.18 mL · min⁻¹ · g⁻¹) and resting vascular resistance (108±17 versus 117±22 mm Hg · mL⁻¹ · min⁻¹ · g⁻¹) were similar on both investigation days.

After saline infusion, adenosine increased perfusion to 1.90±0.33 mL · min⁻¹ · g⁻¹ and reduced vascular resistance to 42±10 mm Hg · mL⁻¹ · min⁻¹ · g⁻¹ (Figure 3A and 3B). After L-NAME infusion, adenosine increased perfusion only to 1.50±0.55 mL · min⁻¹ · g⁻¹ (P<0.01 compared with saline, Figure 3A), and vascular resistance was reduced to only 68±28 mm Hg · mL⁻¹ · min⁻¹ · g⁻¹ (P<0.01 compared with saline, Figure 3B).

On the day of saline treatment, phenolamine infusion did not affect adenosine-induced perfusion or vascular resistance (Figure 3A and 3B). Thus, adenosine-induced perfusion reached 2.10±0.34 mL · min⁻¹ · g⁻¹ (P=NS versus adenosine alone), and vascular resistance reached 37±8 mm Hg · mL⁻¹ · min⁻¹ · g⁻¹ (P=NS versus adenosine alone). On the day of L-NAME treatment, however, phenolamine increased adenosine-induced perfusion to 2.05±0.44 mL · min⁻¹ · g⁻¹ (P<0.01 versus adenosine alone, Figure 3A) and reduced vascular resistance to 45±14 mm Hg · mL⁻¹ · min⁻¹ · g⁻¹ (P<0.01 versus adenosine alone, Figure 3B). Thus, after treatment with phenolamine, the effects of L-NAME on adenosine-induced perfusion and vascular resistance had disappeared.

On the day of saline treatment, adenosine-induced myocardial perfusion reserve and vascular resistance index were not significantly influenced by phenolamine (Figure 4A and 4B). On the day of L-NAME treatment, however, phenolamine significantly increased adenosine-induced perfusion reserve and reduced vascular resistance index (Figure 4A and 4B).

Correlations and Safety

Resting perfusion correlated closely with the rate-pressure product (r=0.92 and 0.75 for protocols 1 and 2, respectively, P<0.01, data not shown).

After the infusion of L-NAME, all participants experienced an unspecific feeling of tiredness, which disappeared during the subsequent infusion of L-arginine. No headache, nausea, ECG abnormalities, or other adverse effects were observed.

Discussion

The major new finding in the present study is that adenosine-induced myocardial hyperemia in humans is partly dependent on endothelial NO production. α-Adrenergic blockade has no effect on adenosine-induced hyperemia unless NO synthesis is inhibited.
It is evident that a tonic vasodilation related to endothelial NO production contributes to the normal regulation of limb blood flow. The role of NO in adenosine-induced vasodilation in the forearm vascular bed, however, is unclear. Some investigators have suggested adenosine-induced hyperemia to be unchanged by NO synthase inhibition, whereas others have reported adenosine-induced hyperemia to be partly dependent on endothelial NO production. In parallel to the findings in the forearm, NO production is important for the regulation of coronary vascular tone, because intracoronary infusion of the NO synthase inhibitor monomethyl-L-arginine (L-NMMA) decreases coronary flow.

In the present study, adenosine-induced myocardial hyperemia has been studied for the first time after intravenous administration of the NO synthase inhibitor L-NAME. A recent study demonstrated that L-NAME causes ~70% inhibition of stimulated NO production in human muscle, as measured by the in vitro conversion of L-arginine to L-citrulline in homogenized biopsies, and also causes significantly higher and more sustained increases in blood pressure than the previously used L-NMMA, indicating effective NO synthase inhibition. The attenuation of the adenosine-induced increase in myocardial perfusion in the present study documents for the first time in humans that adenosine-induced myocardial hyperemia is partly dependent on endogenous NO production.

In animals, α-adrenergic vasoconstrictor activity, for example during exercise, has been suggested to limit coronary perfusion in situations with impaired endothelial function. In humans, the existence and importance of a coronary α-adrenergic vasoconstrictor component has long been debated. Reflex increases in sympathetic activity induced by the cold pressor test are accompanied by vasoconstriction of the coronary arteries as determined by intracoronary Doppler ultrasound measurements in hypertensive but not normotensive individuals. In patients with coronary atherosclerosis, exaggerated vasoconstrictor responses to α-agonists have been documented in arteries, which also show a reduced dilation to endothelium-dependent agonists. These results show that impairment of endothelial vasodilator function may unmask or augment coronary vasoconstriction mediated through α-adrenergic mechanisms.

During L-NAME blockade, adenosine in the presence of phentolamine markedly reduced myocardial vascular resistance compared with adenosine alone. This finding suggests that a certain degree of α-adrenergic vasoconstriction is present during adenosine-induced hyperemia when NO synthesis is inhibited. The reason for this is not clear, because
adenosine does not directly mediate α-adrenergic vasoconstriction. Through several indirect mechanisms, however, adenosine may increase sympathetic activity, for example as a result of activation of chemoreceptors. Furthermore, the adenosine-induced increase in heart rate is known to be accompanied by skeletal muscle sympathetic activity. Such an adenosine-induced increase in sympathetic nervous activity might be particularly marked with the large dose of adenosine used in our study. This is in contrast to studies in which small amounts of adenosine have been administered directly into the coronary arteries, not yielding systemic concentrations high enough to increase sympathetic nervous activity. Therefore, a low level of sympathetic nervous activity could be an explanation for the lack of effect of NO synthase inhibition on adenosine-induced coronary vascular resistance observed in these studies.

In a previous study, α-adrenoceptor blockade with oral doxazosin increased diprydamole-induced myocardial hyperemia. In the present study, there was only a slight and nonsignificant increase in adenosine-induced myocardial hyperemia during phentolamine without concomitant blockade of NO synthesis. Compared with the aforementioned study, our study subjects were considerably younger, and a potential explanation for the divergent results could be age-related differences in endothelial function. The lack of effect of phentolamine on hyperemia suggests that in these young individuals, adenosine is an effective coronary vasodilator despite any reflex sympathetic activation caused by the drug itself.

Important questions concern the mechanisms behind the effect of adenosine at the level of the myocardial resistance vessels and whether NO affects α-adrenergic activity. Our study does not reveal mechanisms at the cellular level, but there are several putative underlying mechanisms that are not mutually exclusive. First, the systemic adenosine-induced vasodilation and increase in perfusion is likely to induce increased NO production in the endothelium. Second, endothelial purinergic receptor stimulation may directly induce NO production in the coronary arteries. Third, the adenosine-induced sympathetic activation could lead to endothelial α2-receptor stimulation, which has been shown to induce NO-dependent vasorelaxation. With the complex interaction of multiple factors regulating blood pressure and heart rate, it is not possible from the present study to identify the exact interaction between α-adrenergic activity and NO. One possible interpretation of our findings is that NO inhibits the effects of α-adrenergic vasoconstrictor activity within the myocardial circulation. A similar role for NO has recently been described in the human skeletal muscle circulation, where NO production during exercise causes local inhibition of the vasoconstrictor activity related to reflex increases in sympathetic activity. Our study puts forward the new hypothesis that NO may also be involved in the regulation of sympathetic vasoconstrictor activity within the myocardium.

Methodological Considerations
PET with [13N]ammonia as flow tracer is a well-established technique with a high degree of reproducibility, which is also evidenced by the similar resting myocardial perfusion values obtained in our study subjects on different days. The effect of pharmacological interventions on blood pressure and heart rate may affect myocardial perfusion and should therefore be considered: (1) L-NAME causes bradycardia, and the adenosine- and phentolamine-induced increases in heart rate were dramatically attenuated after L-NAME compared with saline. In previous studies, however, increasing heart rates from normal levels to 120 bpm by atrial pacing did not significantly change hyperemic coronary blood flow. (2) L-NAME increases blood pressure and thereby perfusion pressure across the myocardial vascular bed, which by itself could lead to an increased hyperemic response. Because L-NAME induced a decrease, rather than an increase, of the adenosine-induced hyperemia, however, either NO synthase inhibition has a direct vasoconstrictor effect within the coronary circulation or the increased pressure causes a myogenic response. (3) Because phentolamine did not change arterial blood pressure in L-NAME–treated individuals, it is unlikely that the substantial increase in adenosine-induced perfusion after α-receptor blockade could be explained by alterations in afterload, but rather it must be due to vasodilation of the myocardial microcirculation. In conclusion, changes in afterload or heart rate are not responsible for the effects for L-NAME and phentolamine on hyperemic myocardial perfusion.

Conclusions
The present study, in healthy humans, demonstrates that adenosine-induced myocardial hyperemia is partly dependent on an intact endogenous NO production and suggests that adenosine-mediated vasodilation is partly endothelium dependent. Thus, a decrease in myocardial perfusion reserve may be caused by endothelial dysfunction and in particular a deficient NO production.

Acknowledgments
This study was supported by the Danish Heart Foundation (grant number 00-1-3-39-22802), Technican Karin Boisen, Department of Cardiology, and technicians at the PET Center are thanked for their assistance during the PET studies.

References


Influence of Nitric Oxide Synthase and Adrenergic Inhibition on Adenosine-Induced Myocardial Hyperemia

Niels H. Buus, Morten Böttcher, Flemming Hermansen, Mikael Sander, Torsten T. Nielsen and Michael J. Mulvany

Circulation. 2001;104:2305-2310
doi: 10.1161/01.CIR.104.19.2305

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/104/19/2305