Role of Nitric Oxide in Adenosine-Induced Vasodilation in Humans

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Abstract—Vasodilation is one of the most prominent effects of adenosine and one of the first to be recognized, but its mechanism of action is not completely understood. In particular, there is conflicting information about the potential contribution of endothelial factors. The purpose of this study was to explore the role of nitric oxide in the vasodilatory effect of adenosine. Forearm blood flow responses to intrabrachial adenosine infusion (125 μg/min) were assessed with venous occlusion plethysmography during intrabrachial infusion of saline or the nitric oxide synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA) (12.5 mg/min). Intrabrachial infusions of acetylcholine (50 μg/min) and nitroprusside (3 μg/min) were used as a positive and negative control, respectively. These doses were chosen to produce comparable levels of vasodilation. In a separate study, a second saline infusion was administered instead of L-NMMA to rule out time-related effects. As expected, pretreatment with L-NMMA reduced acetylcholine-induced vasodilation; 50 μg/min acetylcholine increased forearm blood flow by 150±43% and 51±12% during saline and L-NMMA infusion, respectively (P<.01, n=6). In contrast, L-NMMA did not affect the increase in forearm blood flow produced by 3 μg/min nitroprusside (165±30% and 248±41% during saline and L-NMMA, respectively) or adenosine (173±48% and 270±75% during saline and L-NMMA, respectively). On the basis of our observations, we conclude that adenosine-induced vasodilation is not mediated by nitric oxide in the human forearm. (Hypertension. 1998;31:1061-1064.)

Key Words: adenosine ■ blood flow ■ nitric oxide ■ vasodilation ■ acetylcholine ■ nitroprusside

Adenosine mediates important physiological processes responsible for maintaining metabolic balance during exercise and ischemia. This mechanism appears to be particularly important during ischemia in metabolically active tissues, in which endogenous adenosine is released when metabolic demands exceed oxygen supply. Several of the actions of adenosine may contribute to its protective role during ischemia, and its vasodilatory effect is among the most important. However, the precise mechanism by which adenosine produces vasodilation is not completely understood. Adenosine-induced vasodilation can be explained by a direct relaxing action on vascular smooth muscle. It has also been proposed that the endothelium contributes to this effect, but studies that have addressed this possibility have produced conflicting results. Even in studies in which the endothelium appears to participate in adenosine-induced vasodilation, there is no agreement as to whether nitric oxide or other mechanisms are involved.

The goal of this study was to determine whether nitric oxide generation contributes to the vasodilatory effect of adenosine in humans. For this purpose, we examined whether inhibition of nitric oxide synthesis with Nω-monomethyl-L-arginine (L-NMMA) inhibited the increase in forearm blood flow produced by adenosine in the human forearm.

Methods

We studied a total of 11 normal, healthy men aged 18 to 42 years. Subjects were nonsmokers and free of medications, and they abstained from the use of methylxanthines for 72 hours before the experiments. The volunteer subjects gave written informed consent. The protocols approved by the Vanderbilt University Institutional Review Board.

Instrumentation

Subjects were studied in the fasted state and in the supine position. Heart rate was monitored with surface electrocardiography coupled to a rate computer. An indwelling catheter was placed in the left brachial artery for intra-arterial drug administration. Cardiovascular signals were modulated on signal conditioners and displayed on a thermal array recorder (model TA2000; Gould Inc).

Forearm blood flow was determined with venous occlusion arm plethysmography. The subject’s forearm volume was measured with the water displacement method. The measuring cuff was placed around the forearm. The outer surface of this cuff is rigid, but the surface in contact with the arm is compliant to allow the transmission of changes in forearm volume. The cuff was filled with air to a pressure of 4 cm H2O and connected to a differential transducer (Valdyne Engineering Corp). A second cuff was placed around the wrist and transiently inflated to 50 mm Hg above the systolic blood pressure to exclude the hand from blood flow measurements. A third cuff was placed on the arm, at a site proximal to the measuring cuff, and inflated with an automated device (Medical Instruments, University of Iowa Bioengineering [Iowa City]) to a pressure of 40 mm Hg to produce venous occlusion. The proximal cuff was inflated for 4 seconds at 8-second intervals while the pressure inside the measuring cuff was monitored. Throughout the study, the arm
was kept above the level of the right atria, with the aid of a handrest, to collapse forearm veins. Under these conditions, the rate of change in forearm volume induced by venous occlusion, which is reflected as a change in pressure inside the measuring cuff, correlates with arterial blood flow.

Protocol
Subjects were instrumented as described above and allowed to rest in a quiet room for 20 to 30 minutes. Saline was then infused into the left brachial artery at a rate of 0.2 mL/min. After 10 minutes of saline infusion, increasing dosages of the vasodilator acetylcholine, nitroprusside, or adenosine were administered intrabrachially in random order. Acetylcholine (100 μg/mL) was infused at rates of 30, 40, and 50 μg/min (0.3, 0.4, and 0.5 mL/min, respectively). Nitroprusside (5 μg/mL) was infused at rates of 1, 2, and 3 μg/min (0.2, 0.4, and 0.6 mL/min, respectively). Adenosine (625 μg/mL) was infused at rates of 125, 250, and 500 μg/min (0.2, 0.4, and 0.8 mL/min, respectively).

Each dose was infused for 5 minutes. Forearm blood flow was measured before each drug infusion and during the last minute of each dose. Time was allowed between drugs for the forearm blood flow to return to baseline levels. After this experimental protocol, the intrabrachial infusion of saline was replaced by 2.5 mg/min L-NMMA (12.5 mg/mL at 0.2 mL/min). Ten minutes after the start of L-NMMA treatment, we repeated the infusions of acetylcholine, nitroprusside, and adenosine in random order and as described above.

In a separate group of volunteers, a second saline infusion was administered instead of L-NMMA to ensure any potential changes observed during the L-NMMA period were not due to time-related effects.

Drugs and Statistical Analysis
L-NMMA was purchased from Calbiochem-Novabiochem Corp. Sodium nitroprusside was purchased from ESI Pharmaceuticals (Elkins-Sinn, Inc). Acetylcholine (Miochol) was purchased from IOLAB Pharmaceuticals. Adenosine was purchased from Sigma Chemical Co and dissolved in normal saline at a concentration of 6 mg/mL. The solution was tested for sterility and pyrogenicity.

Data were analyzed using the Number Cruncher Statistical System (NCSS). Statistical evaluation was performed with ANOVA with repeated measures within subjects for multiple comparisons. One factor was the intervention (fixed, two levels: saline and L-NMMA). The second factor was the drugs used (fixed, three levels: adenosine, nitroprusside, and acetylcholine). The third factor was the different dosages of each drug that were used. Values of $P<.05$ were considered significant. Results are expressed as mean±SEM.

Results
We compared the effect of L-NMMA on the vasodilatory actions of adenosine with those of acetylcholine (used as a positive control) and nitroprusside (used as a negative control). The 30-μg/min acetylcholine dosage did not produce consistent increases in forearm blood flow and therefore was not included in the analysis. All other dosages produced significant forearm vasodilation ($P<.001$ for adenosine and nitroprusside and $P=.01$ for acetylcholine by ANOVA; Table). Intrabrachial infusion of L-NMMA reduced resting forearm blood flow from 4.0±0.5 to 2.6±0.2 mL/100 mL of forearm volume per minute. This phenomenon has been reported in previous studies in which this compound was used9 and probably represents inhibition of tonic release of nitric oxide. For this reason, results are expressed as percent change from baseline. L-NMMA had no significant effect on arterial blood pressure. L-NMMA blunted the vasodilatory effect of acetylcholine ($P=.02$ by ANOVA) but not the vasodilatory effect of nitroprusside or adenosine (Table).

Because of the different potencies of these vasodilators, we also compared them at dosages that produced a similar degree of vasodilation: 50 μg/min for acetylcholine, 3 μg/min for nitroprusside, and 125 μg/min for adenosine. Fig 1 presents the absolute values of forearm blood flow at rest and after intrabrachial infusion of 500 μg/min adenosine. L-NMMA had no significant effect on the increase in forearm blood flow produced by adenosine. Adenosine at 500 μg/min increased forearm blood flow from 4.5±0.6 to 23.5±7.5 mL/100 mL of forearm volume per minute during saline infusion and from 2.6±0.2 to 23.6±7 mL/100 mL of forearm volume per minute during L-NMMA infusion.

Acetylcholine at 50 μg/min increased forearm blood flow by 150±43% during saline infusion and by 51±12% during L-NMMA infusion ($P<.01$, n=6). L-NMMA had no significant effect in the increase in forearm blood flow produced by 3 μg/min nitroprusside (165±30% and 248±41% during infusion of saline and L-NMMA, respectively) or 125 μg/min adenosine (173±48% and 270±7% during infusion of saline and L-NMMA, respectively) (Fig 2).

Figure 1. Forearm blood flow (FBF) measured during resting conditions (solid bars) and during intrabrachial infusion of 500 μg/min adenosine (hatched bars) during simultaneous intrabrachial infusion of saline or 2.5 mg/min L-NMMA (n=6).
Figure 2. Increase in forearm blood flow (FBF) produced by intrabrachial infusion of 125 μg/min adenosine (ADO), 3 μg/min nitroprusside (NTP), or 50 μg/min acetylcholine (ACH) during simultaneous intrabrachial infusion of saline (solid bars) or L-NMMA (hatched bars) at 2.5 mg/min (n=6). *P<.01 by ANOVA for the difference between saline and L-NMMA. FBF is expressed as percent change from the preceding resting period.

Because the L-NMMA treatment period was always preceded by a saline treatment period, we studied a separate group of volunteers to determine potential time-related effects that could alter the reproducibility of the vasodilatory effects of these compounds. No significant differences in the degree of vasodilation were found between a first and second period of saline intrabrachial administration. Adenosine increased forearm blood flow from 4.8±1.2 to 24.9±4.7 mL/100 mL per minute during the first saline infusion (419% increase) and from 5.1±2.4 to 32.7±5.8 mL/100 mL of forearm volume per minute during the second saline infusion (541% increase).

Discussion

The purpose of this study was to determine the role of endothelium-derived nitric oxide in the vasodilation induced by intravascular adenosine in humans. L-NMMA, a competitive inhibitor of nitric oxide synthase, was used in this study to inhibit the formation of vascular nitric oxide. Acetylcholine has been shown to stimulate nitric oxide release, and this mechanism accounts for its vasodilatory actions. Acetylcholine was therefore used as a positive control to ensure adequate blockade of nitric oxide production. Nitroprusside acts as a direct nitric oxide donor, producing endothelium-independent vasodilation, and was used as a negative control.

L-NMMA significantly reduced the vasodilatory response to acetylcholine, indicating adequate inhibition of nitric oxide synthase. In contrast, the vasodilatory response to neither adenosine nor nitroprusside were inhibited by L-NMMA. We also demonstrated that the responses to adenosine were reproducible, and therefore the results could not be accounted for by a time effect. From these results, we conclude that vasodilation induced by exogenous adenosine is not mediated by nitric oxide in the human forearm.

Based on early in vitro studies, it was concluded that adenosine produces endothelium-independent vasodilation, and adenosine has been used as a prototype endothelium-independent vasodilator in humans. However, more recent studies have challenged this conclusion and have suggested that the endothelium contributes to, or is even essential for, the vasodilatory effects of intravascular adenosine. When adenosine is administered intra-arterially, labeling studies have shown that the adenosine is contained within endothelial cells and very little escapes this endothelium trap to reach the underlying vascular smooth muscle. Similarly, intravascular administration of adenosine linked to macromolecules, and therefore less likely to cross the endothelium, is still able to produce vasodilation.

Results such as these have prompted investigators to readdress the potential contribution of the endothelium to adenosine-induced vasodilation. In vitro studies, however, have yielded conflicting results as to whether the vasodilatory actions of adenosine are different in vascular preparations with intact or denuded endothelium. Some studies have shown decreased vasodilation induced by adenosine in isolated vascular preparations with denuded endothelium, whereas others have not. Evaluation of putative endothelium-dependent vasodilation by adenosine is difficult to make in vascular ring preparations because adenosine will vasodilate preparations with or without endothelium. Other endothelium-dependent vasodilators will constrict vascular smooth muscle in the absence of endothelium, making their distinction easier.

Adenosine may interact with the endothelium to produce vasodilation via release of nitric oxide. This possibility has been studied through inhibition of nitric oxide synthase. These studies also produced disparate results; in some cases, blockade of nitric oxide production attenuates adenosine-induced vasodilation, but in others, no effect is observed. Furthermore, in the same animals, blockade of nitric oxide synthase attenuates adenosine-induced vasodilation in some vascular beds but not in others. To further complicate the issue, other studies have found that adenosine-induced vasodilation is endothelium dependent but is not mediated by nitric oxide. Raising the possibility that other endothelial factors, such as endothelium-derived hyperpolarizing factor, may be involved.

We believe these seemingly contradictory results cannot be totally explained by diversities in study design and methodology but that other more fundamental differences should be considered. Given the diversity of endothelial cell types, it is possible the endothelial vasodilatory responses to adenosine vary among species and even within the same species depending on the vascular bed under study.

Fewer than a handful of studies have explored this issue in humans. Smits et al reported recently that adenosine-induced vasodilation in the human forearm is inhibited by the nitric oxide synthase inhibitor L-NMMA. This discrepancy with our results may be explained by differences in design and conditions of the experiments. Smits et al found that the increase in forearm blood flow produced by exogenous adenosine is reduced during intrabrachial L-NMMA infusion. To account for the baseline change in forearm blood flow produced by the constrictor effect of L-NMMA, they studied a different group of subjects in whom they infused nitroprusside and L-NMMA simultaneously to restore the original baseline forearm blood flow. The vasodilatory effect of adenosine was reevaluated during this combined infusion. We used a different approach to account for the changes in baseline; we compared the effect of L-NMMA on the vasodilatory actions of adenosine, acetylcholine, and nitroprusside. The last two vasodilators were used as a positive control.
and negative control, respectively. In our experiments, the subjects were used as their own control, and all interventions were done during the same study session. Due to the prolonged half-life of L-NMMA, the control saline intrabrachial treatment always was performed first. A potential time effect was addressed by demonstrating the reproducibility of adenosine effects.

It is unclear whether these differences in study design can account for the discrepant results between the study of Smits et al and the present study. Nevertheless, the results of other studies in humans are in agreement with our findings. For example, it has been shown that adenosine-induced coronary vasodilation is preserved in patients with coronary artery disease in whom the endothelium is deficient. Of greater relevance to our results, in another recent study the effect of L-NMMA infused into the left coronary artery was evaluated

This work was supported in part by National Institutes of Health grants RR-00095, HL-56693, and NS-33460 and NASA grant NAS 9-19483.

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*Hypertension*. 1998;31:1061-1064
doi: 10.1161/01.HYP.31.5.1061

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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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