Role of Nitric Oxide in Reactive Hyperemia in Human Forearm Vessels

Tatsuya Tagawa, MD; Tsutomu Imaizumi, MD; Toyonari Endo, MD; Masanari Shiramoto, MD; Yasuhiko Harasawa, MD; Akira Takeshita, MD

Background The role of nitric oxide (NO) in reactive hyperemia (RH) is not well known. We investigated whether NO plays a role in RH in human forearm vessels by examining the effects of \(N^O\)-monomethyl-L-arginine (L-NMMA), a blocker of NO synthesis, on reactive hyperemic flow.

Methods and Results Forearm blood flow (FBF) was measured by strain-gauge plethysmography with a venous occlusion technique. The left brachial artery was cannulated for drug infusion and direct measurement of arterial pressure. To produce RH, blood flow to the forearm was prevented by inflation of a cuff on the upper arm to suprasystolic pressure for intervals of 3 and 10 minutes. After the release of arterial occlusion (AO), FBF was measured every 15 seconds for 3 minutes. Resting FBF was 4.3±0.3 mL·min\(^{-1}\)·100 mL\(^{-1}\) before 3 minutes of AO and 4.1±0.6 mL·min\(^{-1}\)·100 mL\(^{-1}\) before 10 minutes of AO. FBF increased to 32.3±1.9 and 38.2±3.1 mL·min\(^{-1}\)·100 mL\(^{-1}\) immediately after 3 and 10 minutes of AO, respectively, and gradually decayed (n=13). Intra-arterial infusion of L-NMMA (4 \(\mu\)mol/min for 5 minutes) decreased baseline FBF (P<.01) without changes in arterial pressure. L-NMMA did not affect the peak reactive hyperemic FBF after 3 and 10 minutes of AO. L-NMMA significantly decreased total reactive hyperemic flow (flow debt repayment) by 20% to 30% after 3 and 10 minutes of AO. Simultaneous infusion of L-arginine (a precursor of NO) with L-NMMA reversed the effects of L-NMMA.

Conclusions Our results suggest that NO plays a minimal role in vasodilation at peak RH but plays a modest yet significant role in maintaining vasodilation after peak vasodilation. Our results also suggest that reactive hyperemia in human forearms is caused largely by mechanisms other than NO. (Circulation. 1994;90:2285-2290.)

Key Words • endothelium-derived factors • blood pressure • \(N^O\)-monomethyl-L-arginine

Reactive hyperemia after temporary interruption of blood flow is thought to result from an interplay between physical (myogenic) and local metabolic factors.\(^1\) Of the metabolic factors, prostaglandins,\(^2-6\) adenosine,\(^6-8\) and ATP-sensitive potassium channels\(^9-10\) play an important role in reactive hyperemia.

The endothelium plays an important role in control of vascular tone.\(^11\) Nitric oxide (NO) is produced in the process of conversion of L-arginine to L-citrulline by NO synthase in endothelial cells.\(^12-15\) NO is thought by many to be a major component of endothelium-derived relaxing factor (EDRF) and a potent vasodilator. The role of NO in control of vascular tone during reactive hyperemia has been studied only in animals.\(^16,17\) Previous results suggest that in the coronary circulation of the dog, NO does not play a role in peak reactive hyperemia but plays a significant role during the late phase of reactive hyperemia.\(^16\) In the peripheral circulation of the rat skeletal muscle, it has been shown that NO does not play a significant role in peak reactive hyperemia.\(^18,19\) However, little is known about the role of NO in reactive hyperemia at the peak and during the late phase in the peripheral circulation in humans.

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In this study, we examined the role of NO in the control of vascular tone during reactive hyperemia in human forearm vessels by studying the effects of \(N^O\)-monomethyl-L-arginine (L-NMMA), a blocker of NO synthesis, on forearm blood flow during reactive hyperemia.

Methods

General Procedure
Subjects were healthy male students (21 to 28 years old) at the university who volunteered for the study. The protocol was explained, and informed written consent was obtained from each subject. The study was approved by the Ethical Committee for Human Investigation at our institution. The study was done with subjects in a supine position in an air-conditioned room with room temperature of about 25°C to 26°C. Under local anesthesia with 2% procaine, the left brachial artery was cannulated with a 20-gauge intravascular over-the-needle poly(tetrafluoroethylene) cannula (Quick-Cath, Travenol Laboratories, Inc, Baxter Healthcare Corp) for drug infusion. The cannula was connected by a three-way stopcock to a pressure transducer (Viggo-Spectramed) for direct measurement of arterial pressure. The arterial line was kept open by infusion of heparinized saline (0.1 mL/min) when no drug was being administered. Arterial pressure was monitored continuously. Heart rate was obtained by counting pulse rate for a few minutes on arterial pressure recordings.

See p. 2556

Measurements of Forearm Blood Flow
Forearm blood flow was measured with a mercury-in-Silastic strain-gauge plethysmograph with a venous occlusion technique.\(^20,21\) The strain gauge was placed =5 cm below the antecubital crease. Forearm blood flow (mL·min\(^{-1}\)·100 mL forearm\(^{-1}\)) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented.
by inflation of a cuff on the upper arm. The equation of flow measurement is shown as follows:

\[
\text{Forearm blood flow} = \left( \frac{0.064 \times \text{slope} \times \text{paper speed} (\text{cm/min})}{\text{arm circle} (\text{cm}) \times \text{calibration} (\text{mm}) + 1} \right)^2 - 1 \times 100
\]

where slope denotes the rate of the increase in forearm volume.

Calibration of the plethysmograph was carried out by turning the screw of the plethysmograph to shorten the silicone tube while recording the change in the mercury conductance. The pressure in the venous occlusion or congestion of the cuff on the upper arm was 40 mm Hg. Circulation to the hand was arrested by inflation of a cuff around the wrist. The wrist cuff was inflated before the determination of forearm blood flow and continuously throughout the measurements. Forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic plus one third of the pulse pressure in mm Hg) by the forearm blood flow. These values are expressed as units throughout this report. Forearm blood flow was calculated by two authors (T.T. and T.E.) independently, and an average flow was used for analysis. The individual error for measurement of peak reactive hyperemia was 2.8±0.2%.

**Reactive Hyperemia**

To produce reactive hyperemia, blood flow to the forearm was prevented by inflation of the cuff on the upper arm to suprasystolic pressure.22,23 The duration of arterial occlusion was 10 minutes in 13 subjects and 3 minutes in 8 subjects. It has been shown that maximal vasodilation of forearm resistance vessels is achieved during peak reactive hyperemia after 10 minutes of arterial occlusion.22,24-26 After release of arterial occlusion, forearm blood flow was measured at 5 seconds after release and every 15 seconds thereafter for 3 minutes. Forearm blood flow and arterial pressure were monitored and recorded continuously during reactive hyperemia. The peak flow during reactive hyperemia is markedly greater than control blood flow (Fig 1). Therefore, to accurately calculate forearm blood flow during reactive hyperemia, hyperemic flow was recorded at a paper speed of 30 cm/min, which was three times faster than the speed for control flow recordings. Flow debt repayment was defined as the curve of area under the flow versus time during reactive hyperemia above baseline flow for 3 minutes.9,24,27

**Protocols**

**Experiment 1**

After cannulas and a strain-gauge plethysmograph were placed, at least 15 minutes was allowed for subjects to become accustomed to the study conditions before the protocol was begun. Forearm blood flow was measured at rest and during reactive hyperemia for 3 minutes after 10 minutes of arterial occlusion (n=13). After recovery of reactive hyperemia when forearm blood flow had returned to the baseline level, L-NMMA was infused intra-arterially at a dose of 4 µmol/min for 5 minutes while forearm blood flow and arterial pressure were recorded. We have shown previously that this dose of L-NMMA significantly increases forearm vascular resistance but does not alter arterial pressure.28 Immediately after infusion of L-NMMA was stopped, blood flow to the forearm was prevented for 10 minutes. After release of arterial occlusion, forearm blood flow and arterial pressure were recorded for 3 minutes. In 8 of the 13 subjects, we examined whether L-arginine reversed the effects of L-NMMA on forearm blood flow. At least 30 minutes after release of arterial occlusion, L-NMMA (4 µmol/min) and L-arginine (8 mg/min; about 40 µmol/min) were infused simultaneously and intra-arterially for 5 minutes while forearm blood flow and arterial pressure were recorded. Immediately after infusion of L-NMMA with L-arginine was stopped, 10 minutes of arterial occlusion was repeated. After releases of arterial occlusion, forearm blood flow and arterial pressure were recorded for 3 minutes.

**Experiment 2**

In this experiment, 3 minutes of arterial occlusion was applied in another group of subjects (n=8). All procedures and measurements were done in the same way as experiment 1.

**Drugs**

L-NMMA was obtained from Clinalfa AG. It was dissolved in physiological saline immediately before use. For the infusion of L-arginine, commercially available L-arginine solution (0.1 g of L-arginine per milliliter; Morishita Pharmaceutical) was used.

**Statistical Analysis**

Comparisons between variables before and after L-NMMA and those between variables with infusion of only L-NMMA and with infusion of L-NMMA plus L-arginine were performed with the paired t test. The curves of reactive hyperemia before and after infusion of L-NMMA were compared by two-way ANOVA with repeated measures on both factors.29 One factor was time, and the other was before versus after infusion of L-NMMA. The curves with infusion of only L-NMMA and with infusion of L-NMMA plus L-arginine were compared by the same statistical method. One factor was time, and the other was with infusion of only L-NMMA versus with infusion of L-NMMA plus L-arginine. When the overall difference between the two curves in each comparison reached statistical significance, the values at each point along the time were compared by a post hoc t test.29 Variables during reactive hyperemia were also compared using a repeated measures two-way ANOVA.
Table 1. Forearm Hemodynamics Before and After 10 Minutes of Arterial Occlusion

<table>
<thead>
<tr>
<th></th>
<th>At Rest</th>
<th>During Reactive Hyperemia</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>FBF, mL·min⁻¹·100 mL⁻¹</td>
<td>FVR, U</td>
</tr>
<tr>
<td>Control (n=13)</td>
<td>4.1±0.6</td>
<td>27.9±5.6</td>
</tr>
<tr>
<td>L-NMMA (n=13)</td>
<td>2.8±0.4</td>
<td>36.3±5.5*</td>
</tr>
<tr>
<td>L-NMMA (n=8)</td>
<td>2.3±0.3</td>
<td>41.4±7.1</td>
</tr>
<tr>
<td>L-NMMA+L-arginine (n=8)</td>
<td>3.1±0.3‡</td>
<td>30.6±3.6†</td>
</tr>
</tbody>
</table>

FBF indicates forearm blood flow; FVR, forearm vascular resistance; control, during infusion of saline; L-NMMA, after infusion of N⁰-monomethyl-L-arginine; and L-NMMA+L-arginine, after simultaneous infusions of L-NMMA and L-arginine.

*P<.01 vs control; †P<.05 vs L-NMMA; ‡P<.01 vs L-NMMA.

forearm vessels at rest (Tables 1 and 2) and during hyperemia (Figs 2 and 3, right) and reversed the effects of L-NMMA on flow debt repayment (Tables 1 and 2).

Discussion

Although several previous investigators have examined the role of NO in reactive hyperemia in animals, this is the first study in which the role of NO in reactive hyperemia was examined in humans. The major findings of this study are as follows. First, intra-arterial infusion of L-NMMA (a blocker of formation of endothelium-derived NO) decreased forearm blood flow and increased forearm vascular resistance at rest. Second, L-NMMA did not affect peak forearm vasodilation during reactive hyperemia. Third, L-NMMA significantly (by 20% to 30%) decreased flow debt repayment during reactive hyperemia. Fourth, L-arginine reversed the effects of L-NMMA. These results suggest that although NO plays a significant role in control of vascular resistance at rest, the role of NO in control of vascular resistance during peak reactive hyperemia is minimal. Our results also suggest that NO contributes to reactive hyperemia at the mid-to-late phase, but the magnitude of its contribution is small.

Role of NO in Maximal Vasodilation During Reactive Hyperemia

It has been suggested that NO plays a minimal role in peak vasodilation in the coronary circulation of animals. Kostic and Schrader et al. reported in the coronary circulation of the isolated pig heart that nitro-L-arginine methyl ester (an inhibitor of NO synthesis) did not affect maximal blood flow after brief coronary occlusion. Yamabe et al. reported similar findings in the coronary circulation of the dog. In the peripheral

Table 2. Forearm Hemodynamics Before and After 3 Minutes of Arterial Occlusion

<table>
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<tr>
<th></th>
<th>At Rest</th>
<th>During Reactive Hyperemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FBF, mL·min⁻¹·100 mL⁻¹</td>
<td>FVR, U</td>
</tr>
<tr>
<td>Control (n=8)</td>
<td>4.3±0.3</td>
<td>21.3±1.8</td>
</tr>
<tr>
<td>L-NMMA (n=8)</td>
<td>2.8±0.3†</td>
<td>34.9±4.8†</td>
</tr>
<tr>
<td>L-NMMA (n=7)</td>
<td>2.7±0.4</td>
<td>36.4±4.8</td>
</tr>
<tr>
<td>L-NMMA+L-arginine (n=7)</td>
<td>3.7±0.2‡</td>
<td>24.9±2.0‡</td>
</tr>
</tbody>
</table>

FBF indicates forearm blood flow; FVR, forearm vascular resistance; control, during infusion of saline; L-NMMA, after infusion of N⁰-monomethyl-L-arginine; and L-NMMA+L-arginine, after simultaneous infusions of L-NMMA and L-arginine.

*P<.05 vs control; †P<.01 vs control; ‡P<.05 vs L-NMMA; §P<.01 vs L-NMMA.
circulation, it has been shown that in the rat skeletal muscle, L-NMMA did not affect peak arteriolar dilation and flow velocity during peak reactive hyperemia.\textsuperscript{18,19}

In this study, forearm blood flow and vascular resistance at peak reactive hyperemia after 3 and 10 minutes of arterial occlusion were not different before and after intra-arterial infusion of L-NMMA. Moreover, L-NMMA did not change the time points at which the peak flow occurred in each subject. Our findings are consistent with those in animals\textsuperscript{16-19} and suggest that NO plays a minimal role in vasodilation at peak reactive hyperemia in the human forearm.

To interpret these results, it is important to consider the efficacy of L-NMMA at the dose used in this study in blocking synthesis of NO in the forearm vessels. We demonstrated in this and a previous study\textsuperscript{28} that L-NMMA at 4 \textmu mol/min caused vasoconstriction at rest, which was reversed by L-arginine. In the previous study, we examined the inhibitory effect of L-NMMA at this dose on endothelium-dependent forearm vasodilation caused by acetylcholine at 4 and 12 \textmu g/min, which increased forearm blood flow to approximately 13 and 30 mL \textbullet min\textsuperscript{-1} \cdot 100 mL\textsuperscript{-1}, respectively, before L-NMMA.\textsuperscript{28} L-NMMA at this dose almost completely inhibited modest vasodilation induced by intra-arterial infusion of acetylcholine at a low dose and inhibited by 50\% vasodilation induced by acetylcholine at a high dose.\textsuperscript{28} Thus, we consider that L-NMMA at this dose should have altered the peak reactive hyperemic flow if NO contributed to peak reactive hyperemia. In fact, L-NMMA did not alter the peak reactive hyperemic flow after 3 minutes of arterial occlusion but attenuated comparable increases in flow at the mid-to-late phase of reactive hyperemia after 10 minutes of arterial occlusion (Figs 2 and 3). These results strongly suggest that failure of L-NMMA to alter vasodilation at peak reactive hyperemia is not due to the inadequate dose of L-NMMA and that NO plays a minimal role at peak reactive hyperemia. We elected not to give higher doses of L-NMMA because of a possible increase in systemic blood pressure and vasoconstriction in other vascular beds, particularly in the brain.\textsuperscript{30,31}

Fig 2. Line graphs show forearm blood flow (FBF) at rest and during reactive hyperemia after 10 minutes of arterial occlusion. Left, Results before (c) and after (e) (n=13) N\textdegree-monomethyl-L-arginine (L-NMMA). Right, Results after L-NMMA alone (e) and after L-NMMA plus L-arginine (c) (n=8). L-NMMA decreased baseline blood flow and blood flows during the mid-to-late phase of reactive hyperemia (left). Simultaneous infusions of L-arginine and L-NMMA reversed the effect of L-NMMA (right). *P<.05; **P<.01.

Fig 3. Line graphs show forearm blood flow (FBF) at rest and during reactive hyperemia after 3 minutes of arterial occlusion. Left, Results before (c) and after (e) (n=8) N\textdegree-monomethyl-L-arginine (L-NMMA). Right, Results after L-NMMA alone (e) and after L-NMMA plus L-arginine (c) (n=7). L-NMMA decreased baseline blood flow and blood flows during the mid-to-late phase of reactive hyperemia (left). Simultaneous infusions of L-arginine and L-NMMA reversed the effect of L-NMMA (right). *P<.05; **P<.01.
Although we did not examine the duration of action of intra-arterially infused L-NMMA, a previous study by Vallance et al.\(^{12}\) demonstrated that the increase of forearm vascular resistance induced by intra-arterial infusion of L-NMMA lasted for at least 30 minutes after infusion of L-NMMA was stopped. Thus, we consider that the effects of L-NMMA lasted long enough to block the formation of NO during reactive hyperemia.

The magnitude of peak reactive hyperemic flow depends on the perfusion pressure; ie, the higher perfusion pressure causes a greater reactive hyperemic flow. If intra-arterially infused L-NMMA had increased the perfusion pressure by its systemic effect, an increase in the perfusion pressure would have masked the direct effect of L-NMMA on the reactive hyperemic flow. However, this possibility is unlikely because intra-arterially infused L-NMMA did not alter arterial pressure.

Taken together, we may conclude that NO plays a minimal role, if any, in peak reactive hyperemia in human forearm vessels.

**Role of NO During the Mid-to-Late Phase of Reactive Hyperemia in Humans**

It has been suggested that in the coronary circulation of animals, NO plays a significant role during the late phase of reactive hyperemia. Kostic and Schrader\(^ {17} \) reported that in the coronary circulation of the isolated pig heart, nitro-L-arginine methyl ester reduced flow repayment after brief coronary occlusion. Oxyhemoglobin potentiated the effects of nitro-L-arginine methyl ester.\(^ {17} \) Yamabe et al.\(^ {10} \) reported similar findings in the coronary circulation of the dog.

In this study, L-NMMA reduced blood flow during the mid-to-late phase of reactive hyperemia and decreased flow debt repayment. L-Arginine reversed the inhibitory effect of L-NMMA. It is conceivable that the effect of L-NMMA on forearm blood flow at rest continued unchanged during reactive hyperemia; ie, the attenuated increases in forearm blood flow after L-NMMA during the mid-to-late phase of reactive hyperemia may have been due to decreased forearm blood flow by L-NMMA at rest. However, the differences in forearm blood flow during reactive hyperemia were not only due to the differences in forearm blood flow at rest, because the differences were much greater during reactive hyperemia than at rest. Moreover, flow debt repayment after L-NMMA was smaller than that before L-NMMA. These results suggest that NO plays a significant role in the mid-to-late phase of reactive hyperemia in human forearm vessels.

Flow-dependent vasodilation has been well documented in epicardial coronary arteries,\(^ {33-35} \) mesenteric arteries,\(^ {36} \) and femoral arteries\(^ {37,38} \) in animals and in epicardial coronary arteries in humans.\(^ {39,40} \) Previous studies also have suggested that this flow-dependent vasodilation is caused by the production of NO.\(^ {36,40} \) It has been considered that the increase in flow causes the increase in shear stress that releases NO from the endothelium.\(^ {36,40} \) On the basis of these findings, we assume that the marked increases in forearm blood flow during the early phase of reactive hyperemia might have released NO, which contributed to vasodilation during the mid-to-late phase of reactive hyperemia. The larger decreases in forearm blood flow by L-NMMA during the mid-to-late phase of reactive hyperemia after 10 minutes than after 3 minutes of arterial occlusion may suggest larger amounts of NO release after 10 minutes of arterial occlusion.

**Contribution of Other Factors to Reactive Hyperemia**

Because flow debt was reduced by only about 20% in 10 minutes of arterial occlusion and 25% to 30% in 3 minutes of occlusion by L-NMMA, other mechanisms must dominate, not only in peak dilatation but also in sustained dilatation after occlusion. There are several potential candidates, including prostaglandins, adenosine, and ATP-sensitive potassium channels.\(^ {3-10} \) Wennmalm and colleagues\(^ {3-6} \) have shown in humans that indomethacin decreased the peak level of reactive hyperemia as well as the duration of the hyperemia and that total reactive hyperemia was limited to about 50% by indomethacin or ibuprofen, which are blockers of prostaglandin synthesis. These results suggest that prostaglandins play a significant role not only in peak vasodilation but also during the mid-to-late phase of reactive hyperemia in human vessels. They have also shown that theophylline, an adenosine receptor antagonist, reduced the total reactive hyperemia by about 35% in human forearm vessels.\(^ {6} \) These results suggest that adenosine plays a significant role in reactive hyperemia in humans. Recent studies have shown that ATP-sensitive potassium channels play a role in reactive hyperemia in the canine coronary circulation.\(^ {9,10} \) Thus, ATP-sensitive potassium channels may play a role in reactive hyperemia in human forearm vessels. However, contributions of prostaglandins, adenosine, or ATP-sensitive potassium channels to reactive hyperemia in the forearm were not examined in this study.

In summary, our results may suggest that the role of NO in peak vasodilation during the early phase of reactive hyperemia is minimal but is modest yet significant during the mid-to-late phase of reactive hyperemia in human forearm vessels.

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