Role of nitric oxide in the regulation of digital pulse volume amplitude in humans

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Nohria, Anju, Marie Gerhard-Herman, Mark A. Creager, Shauna Hurley, Debi Mitra, and Peter Ganz. Role of nitric oxide in the regulation of digital pulse volume amplitude in humans. J Appl Physiol 101: 545–548, 2006. First published April 13, 2006; doi:10.1152/japplphysiol.01285.2005.—Measurement of the increase in digital pulse volume amplitude (PVA) during reactive hyperemia relative to baseline (PVA-RH) is being applied widely as a convenient test of nitric oxide bioavailability. However, evidence linking digital PVA-RH to nitric oxide is currently lacking. Accordingly, we investigated whether nitric oxide is responsible for the increase in digital PVA. During reactive hyperemia, we used a peripheral arterial tonometer to record digital PVA at baseline and during reactive hyperemia. The role of nitric oxide in these responses was investigated in 19 healthy subjects by inhibiting nitric oxide synthesis with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME). Ten subjects underwent the identical protocol with saline and five with phenylephrine, a nongeneric property. Instead of L-NAME, relative to baseline (PVA-RH) is being used increasingly to provide a simple means of assessing endothelial function in humans.

THE ENDOTHELIUM PLAYS AN IMPORTANT role in the regulation of vasomotor tone in the human vasculature (9). One of its most potent vasodilator products is nitric oxide, produced by the endothelial isoform of nitric oxide synthase (2). In addition to stimulating vasodilation, nitric oxide possesses key antithrombogenic properties (9). A deficiency of nitric oxide or endothelial dysfunction is a hallmark of cardiovascular diseases and is predictive of poor clinical outcomes (4, 9, 20). The measurement of digital pulse volume amplitude (PVA) during reactive hyperemia (RH), normalized to its baseline value (PVA-RH) is being used increasingly to provide a simple and convenient test of endothelial function and nitric oxide bioavailability (3, 14, 18). As in other regional circulations, the increase in blood flow that occurs during postocclusion RH is thought to stimulate the production and release of endothelium-derived nitric oxide and cause vasodilation (12, 16). However, the relationship between digital PVA-RH and nitric oxide remains presumptive at this time. The potential link between digital PVA-RH and nitric oxide is based on the observations that diseases that reduce endothelial nitric oxide bioavailability also tend to diminish digital PVA-RH (5, 14). Furthermore, reductions in PVA-RH coexist with evidence of endothelial dysfunction in the coronary (5) and brachial arteries (14) of individuals with atherosclerosis or its risk factors.

The digital vasculature is anatomically complex, consisting of a dual circulation of arteriovenous anastomoses and nutritive vessels (6). Arteriovenous anastomoses are particularly abundant in the fingertips and can greatly vary the amount of blood flow in the digits. Vascular tone in these arteriovenous anastomoses is primarily regulated by the sympathetic nervous system, and nitric oxide only plays a minimal role in the regulation of resting digital blood flow (6, 17). However, the role of nitric oxide in modulating digital vascular function in response to a flow stimulus, such as RH, remains unknown. Accordingly, the present study investigated whether nitric oxide is responsible for the changes in digital PVA seen with RH.

METHODS

Study population. The Human Research Committee at Brigham and Women’s Hospital approved the research protocol. Thirty-three apparently healthy volunteers were recruited by advertisement and gave written, informed consent. They consisted of 19 men and 14 women, aged 19–53 yr. None of the subjects were taking any vasoactive medications before enrollment.

Measurement of PVA. A peripheral arterial tonometer was used to measure PVA in the fingertips of the index finger [Ilmar-Medical, Caesarea, Israel (3, 14, 15, 18)]. The peripheral arterial tonometer apparatus consists of a finger-mounted probe that surrounds the fingertip with an electronically controlled, inflatable, pressurized air cushion confined within a rigid external case (3, 14, 15, 18). The pressure changes within the probe that accompany PVA changes in the fingertip are transmitted to a personal computer where the signal is band-pass filtered (0.3–30 Hz), amplified, displayed, and stored.

PVA was analyzed at rest and during RH. RH was elicited by the release of an upper arm blood pressure cuff inflated above systolic pressure for 5 min. Digital PVA-RH was calculated as the ratio of the average PVA over a 1-min time interval starting 1 min before cuff deflation (RH) divided by the average PVA measured for 1 min before cuff inflation (baseline) (3, 14). The PVA from the index finger of the other, nonischemic hand (which was not subject to RH) was measured by 10.220.33.3 on June 19, 2017 http://jap.physiology.org/ Downloaded from

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nitric oxide synthesis alters PVA at rest and during RH. A 20-gauge cannula was inserted into the brachial artery of the study arm at the beginning of the study. After a period of equilibration to achieve a steady state (typically 30–60 min), PVA was recorded under baseline conditions and during RH. The nitric oxide synthase inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME, Clinalfa, Laufelfingen, Switzerland) (1, 7, 10, 21) was then infused into the brachial artery of the study arm at a dose of 3 μg/min (19) for 10 min using a Harvard pump (Harvard Apparatus, South Natick, MA). PVA was recorded during the first 5 min of the L-NAME infusion. The cuff was reinflated above systolic pressure during the second 5 min of the L-NAME infusion and PVA was determined again after cuff release (RH).

Ten additional subjects were studied in an identical manner with saline infusion instead of L-NAME. Five subjects underwent the identical protocol with infusion of the endothelium-independent vasoconstrictor phenylephrine (1 mcg/min) instead of L-NAME. One subject participated in both the L-NAME and phenylephrine study arms on separate days.

**Statistical analysis.** Data are expressed as means ± SD in Table 1. All other data are expressed as means ± SE. Baseline characteristics between the three groups were compared using ANOVA for continuous variables and Fischer’s exact test for categorical variables. The change in baseline digital PVA and the change in PVA-RH after drug administration in the study arm was compared between the three groups using ANOVA or the Kruskal-Wallis analysis of ranks test based on whether the change was normally distributed or not. Significant differences were then evaluated further by comparing the change in baseline digital PVA and the change in PVA-RH after drug administration, digital PVA-RH in the control arm was analyzed using linear regression analysis with repeated measures. All analyses were performed using SAS software version 8.0 (SAS Institute, Cary, NC). Statistical significance was accepted at \( P \leq 0.05 \).

## RESULTS

**Study population.** The clinical characteristics of the study subjects are presented in Table 1. The subjects were relatively young, apparently healthy, and free of cardiovascular risk factors. The subjects were well matched in all three treatment groups.

**Effect of saline on pulse volume amplitude.** Baseline digital PVA for each subject was assigned a value of 1. PVA increased and typically peaked at 1 min of RH after the release of arm occlusion (Fig. 2). PVA-RH in the study hand increased to 2.04 ± 0.11 (i.e., 105 ± 12% increase in PVA-RH compared with the baseline PVA value). After 5 min of saline infusion, baseline digital PVA in the study hand (pre-RH) decreased by 5 ± 2% to 0.90 ± 0.02 \( (P = \text{not significant (NS)}) \). Digital PVA-RH in the study hand increased by 9 ± 4% from 2.04 ± 0.11 before to 2.14 ± 0.12 after the administration of saline \( (P = 0.05) \) (Fig. 3).

**Effect of L-NAME on PVA.** Baseline digital PVA for each subject was assigned a value of 1. Digital PVA-RH in the study hand before L-NAME infusion was 2.03 ± 0.27. L-NAME decreased baseline (pre-RH) digital PVA in the study hand to 0.95 ± 0.02. This decrease in baseline PVA was similar to that seen in the patients receiving saline \( (P = \text{NS}) \). After L-NAME administration, digital PVA-RH in the study hand was reduced from 2.03 ± 0.27 to 1.58 ± 0.14. Hence, inhibition of nitric oxide synthesis with L-NAME reduced the increase in PVA-RH by 46 ± 21% \( (P = 0.002 \text{ compared with saline}) \) (Fig. 3).

**Effect of phenylephrine on PVA.** Phenylephrine infusion reduced baseline PVA in the study finger by 50 ± 12% \( (\text{from 1.50 ± 0.12; } P = 0.003 \text{ compared with saline}) \). However, digital PVA-RH increased by 20 ± 9% from 1.43 ± 0.14 before to 1.60 ± 0.22 after phenylephrine \( (P = \text{NS compared with saline}) \) (Fig. 3).

**PVA in the control arm.** There was a downward drift over time in the PVA signal of the control finger in all three treatment groups \( (P = 0.02; \text{Table 2}) \). However, the group × time interaction term was not significant, suggesting that the drift was similar in all three treatment groups \( (P = 0.48) \).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Saline ((n = 10))</th>
<th>L-NAME ((n = 19))</th>
<th>Phenylephrine ((n = 5))</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
<td>6/4</td>
<td>11/8</td>
<td>4/1</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
<td>32 ± 4</td>
<td>31 ± 4</td>
<td>25 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>88 ± 10</td>
<td>89 ± 9</td>
<td>75 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Serum cholesterol, mg/dl</td>
<td>171 ± 22</td>
<td>157 ± 15</td>
<td>157 ± 26</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n, \) no. of subjects. L-NAME, \( N^G\text{-nitro-L-arginine methyl ester; NS, not significant.} \) \( P \) value defines comparison between the 3 treatment groups.
DISCUSSION

In the present study, we demonstrate that nitric oxide is the mediator responsible for the marked increase in digital PVA-RH in apparently healthy subjects free of cardiovascular risk factors. By pharmacologically inhibiting nitric oxide synthase, we found that approximately one-half of the increase in digital PVA-RH is mediated by nitric oxide. In contrast, phenylephrine, a vasoconstrictor that acts largely independently of nitric oxide inhibition, reduced resting PVA but had no effect on digital PVA-RH. These results suggest that the observed reduction in digital PVA-RH by l-NAME is specifically mediated by nitric oxide inhibition and not by nonspecific vasoconstriction. Thus, to our knowledge, this is the first study that provides biological validity for the measurement of digital PVA-RH as a test of endothelial function.

Two major factors govern the magnitude of digital PVA during each cardiac cycle: vascular distensibility, which permits additional blood to enter the digit with each cardiac cycle, and the digital blood flow. It is likely that nitric oxide prominently affects the first of these factors during RH in the human finger. Prior investigations have shown that nitric oxide plays a role, although a minimal one, in controlling resting digital blood flow (6, 17). In this study, inhibition of nitric oxide synthase did not alter baseline digital PVA and thus our results are in agreement with prior findings. The principal new finding of this study is that nitric oxide is released during RH in the digit, as evidenced by the significant reduction in digital PVA when nitric oxide production is inhibited by l-NAME. Vascular distensibility determines how the augmented blood flow during RH is accommodated with each cardiac cycle. Our laboratory has previously shown in the human brachial artery that vascular distensibility is augmented by endogenous nitric oxide (13). Hence, the present observations in the digital circulation taken together with prior studies of other human vascular beds strongly suggest that the dependence of digital PVA-RH on nitric oxide is due to the pivotal role that nitric oxide exerts in augmenting vascular distensibility.

The present study, by defining the central role of nitric oxide in digital PVA-RH, has potentially important clinical implications. It is notable that abnormalities in PVA were used as a marker of cardiovascular disease many years before the pioneering description of nitric oxide as an endothelium-dependent vasodilator by Furchgott and Zawadzki (8). In the “Men Born in 1914” study, PVA-RH was measured in the lower extremities of 636 men from Malmo, Sweden, who had no symptoms or signs of obstructive lower extremity vascular disease (11). Interestingly, a 21-yr follow-up of this cohort revealed that reduced PVA-RH was an independent and strong predictor of increased cardiac events (11). In retrospect, this study corroborates the notion that endothelium-derived nitric oxide exerts in augmenting vascular distensibility.

Table 2. Pulse volume amplitude over time in control arm

<table>
<thead>
<tr>
<th></th>
<th>Predrug Baseline</th>
<th>Predrug RH</th>
<th>Postdrug Baseline</th>
<th>Postdrug RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 10)</td>
<td>1.03 ± 0.02</td>
<td>0.92 ± 0.02</td>
<td>0.87 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>l-NAME (n = 19)</td>
<td>1.05 ± 0.04</td>
<td>0.93 ± 0.01</td>
<td>0.89 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Phenylephrine (n = 5)</td>
<td>0.86 ± 0.06</td>
<td>0.91 ± 0.22</td>
<td>0.83 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. P value not significant for between-group comparison. PVA, pulse volume amplitude; RH, reactive hyperemia in study arm.
Nitric oxide is an excellent predictor of long-term cardiovascular outcomes (4, 9, 20). More recently, digital PVA-RH measured by peripheral arterial tonometry has been shown to correlate with validated tests of endothelial function in other vascular beds. In particular, digital PVA-RH correlated with flow-mediated dilation in the brachial arteries of patients with cardiovascular risk factors (14) and with acetylcholine responses in the coronary arteries of patients referred for angiography (5). As expected from our results, digital PVA-RH is blunted in patients with coronary disease or its risk factors, common disease processes associated with endothelial dysfunction, and loss of nitric oxide (5, 14). Conversely, in patients with symptomatic coronary disease, digital PVA-RH is increased by a therapy that appears to improve cardiovascular status: enhanced external counterpulsation (3). This body of evidence, indirectly, and the present study, directly, support the central involvement of nitric oxide in augmenting digital PVA-RH. Furthermore, because the inhibition of nitric oxide blunted the increase in PVA-RH from 104 to 58%, this assessment of nitric oxide bioavailability has a particularly broad and hence favorable dynamic range. Coupled with the relative simplicity of the measurement, the present study suggests that measurement of digital PVA-RH may prove to be a useful test of nitric oxide bioavailability and endothelial function.

Potential limitations. The human finger has an anatomically dual circulation consisting of nutritive vessels and arteriovenous anastomoses. Our experiments used methods to assess total digital PVA. Hence this study was not designed to clarify the respective role that each of these two circulations plays in augmenting PVA-RH. We cannot ascertain whether the drugs reached the fingers in full concentrations while the upper arm was occluded by the blood pressure cuff to induce RH. In as much as we “only” inhibited the PVA-RH by 46% with L-NAME, the implication is the inhibition may have been more had L-NAME fully reached the fingers. The alternate postulate that the drug may have reached higher than intended concentrations because the venous outflow from the hand was also interrupted by the cuff inflation would only be relevant if the drug had some additional properties at higher than intended concentrations. We are unaware of any such properties.

In summary, the present study has established an important role for nitric oxide in the increase in digital PVA-RH. This straightforward measurement in the fingertip may provide a simple means of assessing nitric oxide bioavailability as a test of endothelial function in humans.

GRANTS
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REFERENCES