Endothelium-Derived Nitric Oxide Contributes to the Regulation of Venous Tone in Humans

Daniel J. Blackman, MRCP; Jayne A. Morris-Thurgood, PhD; John J. Atherton, FRACP; Gethin R. Ellis, MRCP; Richard A. Anderson, MRCP; John R. Cockcroft, MD, FRCP; Michael P. Frenneaux, MD, FRCP

Background—Although nitric oxide (NO) is known to play an important part in the regulation of arterial tone, little is known about its role in veins. The aim of this study was to investigate the role of basal and stimulated NO activity in the regulation of tone of the human venous capacitance bed.

Methods and Results—We measured venous tone using radionuclide forearm venous plethysmography in 24 healthy subjects with no cardiovascular risk factors. In 13 subjects, basal NO activity was assessed by measuring the effects on venous tone of an intra-arterial infusion of the NO synthase inhibitor N-monomethyl-L-arginine (L-NMMA). In the remaining 11 subjects, stimulated NO activity was evaluated by measuring the effects of an intra-arterial infusion of incremenal doses of carbachol, followed in a subgroup by coinfusion with L-NMMA. Infusion of carbachol caused dose-dependent venodilation, with a maximal reduction in forearm venous tone of 40.1±12.5% (P<0.0001). Carbachol-induced venodilation was inhibited by L-NMMA (48.9±6.2% reversal of maximal venodilation, P<0.01). Infusion of L-NMMA alone caused vеноconstriction (9.1±6.4% increase in venous tone, P=0.002).

Conclusions—Human forearm capacitance veins exhibit both stimulated and basal NO activity, which indicates that NO contributes not only to the regulation of venous tone but also to resting venous tone in healthy human subjects. (Circulation. 2000;101:165-170.)

Key Words: veins • endothelium • nitric oxide

In the arterial circulation, release of endothelium-derived nitric oxide (NO) is stimulated by shear stress and by agonists such as acetylcholine, carbachol, and bradykinin.1–4 Basal NO activity has also been demonstrated by the observation of vasoconstriction in response to N-monomethyl-L-arginine (L-NMMA), a selective inhibitor of NO synthase.5 These findings indicate that NO is involved in the regulation of arterial tone and contributes to resting tone.

The role of NO in the regulation of venous tone is less clear. Animal studies in vivo and in vitro have shown marked heterogeneity in stimulated NO activity between different venous preparations.6,7 Evidence of NO activity in humans has been limited to work in conduit veins (superficial hand veins and saphenous vein grafts).8–11 These studies have suggested that although NO activity can be stimulated by endothelium-dependent agonists, basal NO activity is absent, and therefore NO does not contribute to resting venous tone. However, no study in humans has investigated the small veins and venules of the capacitance bed, which are predominately responsible for overall venous tone. Furthermore, recent indirect evidence from animal studies suggests that basal NO activity may contribute to tone in the venous capacitance bed.12

The principal aim of this study was to investigate both stimulated and basal NO activity in human capacitance veins to determine the role of endothelium-derived NO in the regulation of venous tone. Venous tone was assessed in the forearm venous capacitance bed of normal subjects by radionuclide plethysmography. Stimulated and basal NO activity were assessed by measuring the effects on venous tone of intra-arterial carbachol and L-NMMA.

Subjects
Twenty-four healthy volunteers with no history of active smoking, hypertension, diabetes mellitus, or hypercholesterolemia and no family history of ischemic heart disease were studied. None of the volunteers were taking cardioactive medication or antioxidant vitamin supplements. All gave written informed consent, and the study was approved by the hospital research and ethics committee.

Measurement of Venous Tone
Venous tone was assessed in the forearm capacitance bed by radionuclide venous plethysmography. This technique involves labeling of red blood cells with 99mTc. At least 90% of the injected isotope is confined to the intravascular space; therefore, forearm radioactive counts are proportional to forearm blood volume. Because the vast majority of blood in the peripheral circulation is contained within the veins,13,14 changes in counts reflect changes in venous volume. Construction of a venous volume-pressure relation allows assessment of venous tone. A parallel shift of the volume-

Received January 13, 1999; revision received August 10, 1999; accepted August 16, 1999.
From the Department of Cardiology, University of Wales College of Medicine, Cardiff, UK.
Correspondence to Professor M.P. Frenneaux, Department of Cardiology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, UK. E-mail daviesja3@cardiff.ac.uk
© 2000 American Heart Association, Inc.
Circulation is available at http://www.circulationaha.org

165
pressure relation implies a change in venous tone. A change in slope indicates altered compliance.

Red cells were labeled by an in vivo method. A cannula was inserted into the antecubital fossa of the dominant arm. Stannous medronate (Amerscan, Amersham UK; 0.03 mL/kg) was injected intravenously, followed 20 minutes later by injection of 750 MBq of $^{99m}$Tc pertechnetate. In the presence of stannous ion, $^{99m}$Tc is reduced within the cells and becomes bound to the $\beta$-chains of the globin.

A sphygmomanometer was placed around the nondominant upper arm. The forearm was positioned comfortably on a face of a 20-cm field-of-view gamma camera equipped with a low-energy, super-high-sensitivity, parallel-hole collimator and with an integrated computer system (Elscint Apex 215M). The static image of the forearm was continuously acquired, and at 90-second intervals, the cuff was inflated to produce venous occlusion pressures of 0, 10, 20, and 30 mm Hg.

After acquisition, a region of interest was defined on the forearm image. The counts in the region of interest were acquired in the final 60 seconds of each 90-second interval. The count rate in the region of interest obtained with no occluding pressure was arbitrarily taken to represent 100% forearm blood volume. All subsequent readings were expressed as a percentage of this value. Means of scintigraphic vascular volumes (in percent units) at occluding cuff pressures of 0, 10, 20, and 30 mm Hg were used to construct venous volume-pressure plots after correction for physical decay.

### Study Protocol

The investigations were performed at the University Hospital of Wales (Cardiff, UK) in a temperature-controlled laboratory (22°C to 24°C). All studies were performed with the subject having fasted and abstained from caffeine-containing drinks for $\geq 6$ hours previously.

After red cells were labeled as described above, a 27-gauge unmounted steel needle (Cooper’s Engineering), sealed with dental wax to an epidural cannula, was inserted into the brachial artery of the nondominant arm under sterile conditions. The arm was then positioned on the gamma camera. To minimize the amount of free circulating $^{99m}$Tc, imaging was commenced $\geq 30$ minutes after initial labeling. Baseline measurements were performed during intra-arterial infusion of 0.9% saline (1 mL/min). Four minutes after infusion of saline commenced, a venous volume-pressure relation was recorded. Changes in unstressed venous volume were determined with linear regression, paired $t$ tests, and ANOVA as appropriate. A value of $P<0.05$ was considered significant.

### Data Analysis

#### Forearm Venous Tone

Venous volume-pressure plots were constructed for each stage in each subject. Linear regression was performed on each set of data points to determine whether a linear model described the data. A linear model was accepted if $r$ was $\geq 0.8$. We then determined whether the slopes of the lines in each data set were different (ie, to determine whether the lines were parallel or not) using a standard method for comparing 2 independent regressions.$^{20}$

Unstressed venous volume was defined as the intercept on the volume axis. Resting unstressed venous volume in each subject was calculated as the mean of the 2 unstressed venous volumes during infusion of normal saline. Changes in unstressed venous volume reflect changes in venous tone. Increases in venous tone are expressed as percentage of venous constriction and decreases in venous tone as percentage of venodilatation.

### Statistical Analysis

Data are expressed as mean±SEM. Statistical analysis was performed with linear regression, paired $t$ tests, and ANOVA as appropriate. A value of $P<0.05$ was considered significant.

### Results

#### Subject Characteristics

The study group consisted of 16 men and 8 women aged 27 to 75 years (mean 50.4 years). All subjects had normal fasting levels of cholesterol, glucose, and homocysteine and were normotensive at rest (Table 1).

#### Changes in Forearm Venous Tone

For each subject, 2 baseline volume-pressure plots were generated during infusion of normal saline and 1 during

---

**TABLE 1. Baseline Characteristics of Subject Group**

<table>
<thead>
<tr>
<th>Demographic and Clinical Features</th>
<th>Demographic and Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, y</strong></td>
<td>50±12</td>
</tr>
<tr>
<td><strong>Sex, M/F, n</strong></td>
<td>16/8</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m$^2$</strong></td>
<td>27.2±2.2</td>
</tr>
<tr>
<td><strong>Smoking history, n</strong></td>
<td>19</td>
</tr>
<tr>
<td><strong>Nonsmoker</strong></td>
<td>5</td>
</tr>
<tr>
<td><strong>Current smoker</strong></td>
<td>0</td>
</tr>
<tr>
<td><strong>Serum cholesterol, mmol/L</strong></td>
<td>5.1±0.7</td>
</tr>
<tr>
<td><strong>Plasma glucose, mmol/L</strong></td>
<td>4.9±0.4</td>
</tr>
<tr>
<td><strong>Plasma homocysteine, mmol/L</strong></td>
<td>7.6±2.4</td>
</tr>
<tr>
<td><strong>Heart rate, min$^{-1}$</strong></td>
<td>63±8</td>
</tr>
<tr>
<td><strong>Blood pressure, mm Hg</strong></td>
<td>136±12</td>
</tr>
<tr>
<td><strong>Systolic</strong></td>
<td>74±8</td>
</tr>
</tbody>
</table>

Continuous data are presented as mean±SEM.
infusion of each dose of carbachol or L-NMMA. Linear regression was performed for each plot, and \( r \) values were 0.81 to 0.99 (mean 0.94±0.04). Therefore, a linear model was adopted. Although volume-pressure plots varied in slope (compliance) between individuals, within individuals there was little change in slope at different stages of the study. In other words, shifts in the plots induced by infusion of the active agents were parallel and were due to changes in venous tone rather than in compliance.

**Response to Carbachol**

Infusion of carbachol caused a dose-dependent parallel upward shift in the volume-pressure relation, with an increase in unstressed venous volume, consistent with venodilation (Figure 1). The maximum administered dose (15 \( \mu \)g/min) produced a 40.1±12.5% reduction in venous tone (\( P<0.0001 \)) (Figure 2a). In the subgroup of 4 subjects who received coinfusion of carbachol with L-NMMA, the maximum venodilator response was reduced by 48.9±6.2% to 17.6±4.5% (\( P<0.01 \)) (Figure 2b).

**Response to L-NMMA**

The response to L-NMMA is illustrated in Figures 1c and 2c. Infusion of L-NMMA alone resulted in 9.1±6.4% vеноconstriction (\( P=0.002 \)), which demonstrates that basal NO activity was responsible for an average reduction of \( \approx10\% \) in resting venous tone in this cohort of healthy volunteers.

**Response to Hydralazine**

Hydralazine caused progressive arterial vasodilation, with a peak increase in FBF of 262±102% at 30 minutes (\( P<0.01 \)) (Table 2). This was not associated with any significant change in unstressed venous volume (Figure 1d).
The important new finding of this study is that healthy subjects exhibit both stimulated and basal NO activity in the forearm venous capacitance bed, which indicates that endothelium-derived NO is involved in the regulation of venous tone and that it contributes to resting venous tone. This may have important implications in chronic heart failure (CHF), in which venous endothelial dysfunction and reduced NO activity might contribute to the increase in venous tone that characterizes this condition,21 and raises the possibility that novel therapeutic strategies might be devised that improve endothelial function and thereby reduce venous tone in CHF.

Role of NO in the Regulation of Vascular Tone

The importance of the endothelium and NO in the arterial circulation is well recognized. In addition to antiatherogenic effects (such as inhibition of platelet aggregation and vascular smooth muscle cell proliferation), NO is a potent vasodilator.22–25 Through both stimulated and basal activity, NO contributes importantly to the regulation of arterial tone.5 The role of NO in the regulation of venous tone is less well understood. Previous work in humans has been limited to studies of superficial hand veins and saphenous vein (SV) grafts. Vallance and colleagues8 measured the effects of intravenous infusions of acetylcholine and L-NMMA on the diameter of dorsal hand veins in healthy subjects. In veins preconstricted with noradrenaline, low-dose acetylcholine caused dilation, which was almost eliminated by L-NMMA. However, infusion of L-NMMA alone had no effect on vein diameter. They therefore concluded that although veins are capable of producing NO in response to agonists, basal NO activity is absent. These findings are supported by a number of organ bath experiments that contrast NO activity in internal mammary artery (IMA) and SV grafts,9 –11 although it should be emphasised that these studies were performed in patients undergoing CABG surgery, who therefore would be likely to have endothelial dysfunction. In one particularly interesting study by Hamilton et al,9 carbachol produced relaxation of both IMA and SV rings, although this was attenuated in the veins. The NO synthase inhibitor N\textsuperscript{G}-nitro-L-arginine (L-NAME) caused an increase in tension in IMA rings but had no significant effect in SV rings. However, when these veins were pretreated with the intracellular free radical scavenger tiron, L-NAME caused a 47% increase in tension. In the same study, the authors investigated endothelial NO synthase (eNOS) activity using immunocytochemical techniques; eNOS positivity was present in the SVs but much less intense than in the IMAs. Taken together, these findings suggest that basal NO activity is absent.

![Figure 2](http://circ.ahajournals.org/)

**Figure 2.** Changes in venous tone induced by infusion of carbachol (a), carbachol and L-NMMA coinfusion (b), and infusion of L-NMMA alone (c). a, Carbachol caused dose-dependent venodilation (\(\*P<0.05\), †\(P<0.01\), ‡\(P<0.005\), §\(P<0.0001\)). b, After coinfusion with L-NMMA, there was inhibition of this effect, which confirms that carbachol causes stimulated NO activity (†\(P<0.01\) vs carbachol alone). c, Infusion of L-NMMA alone produced venuconstriction, which indicates basal NO activity (‡\(P<0.005\)).
Results of animal studies have suggested that NO activity is heterogenous between different venous preparations. For example, Vedernikov et al. demonstrated marked endothelium-dependent relaxation in jugular, femoral, and mesenteric veins but found this was absent in the saphenous, portal, and inferior caval veins, despite preservation of endothelium-independent relaxation in response to sodium nitroprusside.

In view of such variability in NO activity in different venous beds, we believed that findings in human conduit veins could not be presumed to reflect the physiologically much more important capacitance veins. Furthermore, recent animal studies suggest that basal NO activity may contribute to resting venous tone. In a study of awake and instrumented rats, L-NMMA caused a dose-dependent increase in mean circulatory filling pressure that was reversed by the administration of L-arginine.

Validation of Technique

We assessed venous tone in the forearm capacitance bed using radionuclide venous plethysmography. This technique was first described by Rutlen in 1981. It has been validated against assessment of venous volume by both strain-gauge and fluid-displacement plethysmography and has been found to be highly reproducible. Subsequently, it has been used to assess regional venous volume and venous tone in a number of studies. In the present study, we found that intra-arterial infusion of the selective arterial vasodilator hydralazine had no effect on unstressed venous volume measured by radionuclide plethysmography despite the fact that it caused a considerable increase in arterial inflow. This confirms that the shifts in the venous volume-pressure relation produced by carbachol and L-NMMA must have been due to changes in venous tone and not merely to a reflection of their effects on arterial inflow. This is further supported by the previous demonstration by Manyari et al. of an upward shift in the venous volume-pressure relation consistent with venodilation in response to sublingual glyceryl trinitrate but no such effect after sublingual administration of the selective arterial vasodilator nifedipine.

We have confirmed previous studies in human conduit veins in demonstrating dose-dependent venodilation in response to the agonist carbachol. Additionally, we have shown that this response is inhibited by L-NMMA, which indicates that it is NO dependent. That reversal of carbachol-induced venodilation with L-NMMA was only partial is consistent with the findings of studies in the arterial circulation and reflects increasing evidence that agonist-induced endothelium-dependent dilatation is not solely mediated by NO but also involves other mechanisms, including as-yet-unidentified endothelium-dependent hyperpolarizing factors.

We have also demonstrated basal NO activity in human capacitance veins, which indicates that NO contributes to resting venous tone. This is in marked contrast to the only other studies of the human venous endothelium in healthy subjects, namely, those in superficial hand veins, and suggests an important difference in the behavior of the conduit veins of the hand and the capacitance veins of the forearm. This is consistent with the findings of animal studies that demonstrated heterogeneity between different venous preparations. It is the veins of the capacitance bed that predominantly determine overall venous tone. Therefore, our finding of both stimulated and basal NO activity in capacitance veins provides compelling new evidence that NO contributes to the regulation of venous tone and to resting venous tone in human health.

We did not detect significant changes in the slopes of the venous volume-pressure relations after infusion of either carbachol or L-NMMA, which indicates that alterations in NO activity do not affect compliance in the forearm venous capacitance bed. Although our findings are in contrast to the arterial circulation, in which NO appears to play an important role in the regulation of compliance, they parallel those of a number of other studies in which the NO donor glyceryl trinitrate caused a reduction in tone of the venous capacitance bed with no effect on compliance. Furthermore these results support the generally held concept that compliance remains relatively constant in the venous bed, whereas changes in unstressed volume are the major determinant of capacitance.

Clinical Implications

The majority of blood volume lies within the venous capacitance bed; hence, small changes in venous tone may translocate relatively large volumes of blood to or from the central compartment. Such shifts in central blood volume will alter right ventricular and consequently left ventricular end-diastolic volume and will as a result, via the Frank-Starling mechanism, affect stroke work. Our observations therefore suggest that the venous endothelium, via its effect on venous tone, may significantly influence stroke work and hence cardiac output. This may have particular relevance in CHF, in which increased central blood volume, mediated in part by increased venous tone, appears to have a deleterious effect on cardiac performance. If endothelial dysfunction contributes to increased venous tone in CHF, agents targeted at improving endothelial function may provide a novel strategy for venodilator therapy.

Study Limitations

Potential limitations of this study relate to the technique of radionuclide forearm venous plethysmography and its application in the measurement of venous tone. The technique uses radioactive counts within a predefined region of interest to represent forearm venous volume. However, a proportion of labeled red blood cells will be intra-arterial rather than within the venous system, so that the translation of forearm counts to forearm venous volume is not absolutely accurate. Previous studies have estimated that 70% to 80% of total intravascular volume resides within the venous system. In a peripheral vascular bed such as the forearm, this proportion is likely to be even higher. It is therefore unlikely that the changes in blood volume seen in this study could be due primarily to changes in arterial volume. Furthermore, we have demonstrated that infusion of the selective arterial vasodilator hydralazine had no effect on forearm vascular volume as measured by this technique.

Validation of the radionuclide plethysmography technique by intra-arterial infusion of hydralazine was performed in only 5 subjects. Because the results of these studies demonstrated consistently that a huge increase in arterial inflow had no effect on the venous volume-pressure relation, and furthermore because these findings were in accord with those of the study by Manyari et al. using sublingual nifedipine, we felt that greater numbers were not required. Similarly, dem-
onstration of inhibition of carbachol-induced venodilation with L-NMMA was performed in only 4 volunteers. However, these studies were also consistent in their findings, with very little variability between subjects, and were performed primarily to confirm previous observations, in both arteries and veins, that muscarinic agonist–induced endothelium-dependent dilation is, at least in part, NO dependent.\(^1\),\(^8\),\(^30\)

We have investigated responses in the forearm venous capacitance bed alone, which will not necessarily be mirrored in other capacitance beds, such as the quantitatively more important splanchnic and splenic venous beds. Assessment of these beds in humans, although possible,\(^34\) would be problematic and would require invasive techniques to assess the endothelium.

**Conclusions**

Human forearm capacitance veins exhibit both stimulated and basal NO activity, which indicates that NO has an important role in the regulation of venous tone and contributes to resting venous tone in healthy human subjects.

**Acknowledgments**

Dr Blackman, Dr Morris-Thurgood, Dr Ellis, Dr Anderson, and Professor Frenneaux are supported by the British Heart Foundation. We are grateful to Dr E.A. Jones, Department of Medical Physics, University Hospital of Wales, for her assistance with the radionuclide plethysmography technique.

**References**


11. Yang Z, von Segesser L, Bauer E, Stulz P, Turina M, Lüscher TF. Different basal NO activity, which indicates that NO has an important role in the regulation of venous tone and contributes to resting venous tone in healthy human subjects.
Endothelium-Derived Nitric Oxide Contributes to the Regulation of Venous Tone in Humans


Circulation. 2000;101:165-170
doi: 10.1161/01.CIR.101.2.165

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 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/101/2/165

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/