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Enhanced Release of Prostaglandins Contributes to Flow-Induced Arteriolar Dilation in eNOS Knockout Mice

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Abstract—Nitric oxide and prostaglandins were shown to contribute to the endothelial mediation of flow-induced dilation of skeletal muscle arterioles of rats. Thus, we hypothesized that flow-induced dilation and its mediation are altered in gracilis muscle arterioles of mice deficient in the gene for endothelial nitric oxide synthase (eNOS-KO) compared with control wild-type (WT) mice. Gracilis muscle arterioles (≈80 μm) of male mice were isolated, then cannulated and pressurized in a vessel chamber. The increases in diameter elicited by increases in perfusate flow from 0 to 10 μL/min were similar in arterioles from eNOS-KO (n = 28) and WT (n = 22) mice (≈20 μm at 10 μL/min flow). Removal of the endothelium eliminated flow-induced dilations in vessels of both strains of mice. Nw-nitro-L-arginine (L-NNA, 10⁻⁴ mol/L) significantly inhibited flow-induced dilation in arterioles of WT mice by ≈51% but had no effect on responses of arterioles from eNOS-KO mice. Indomethacin (INDO, 10⁻⁵ mol/L) inhibited flow-induced dilation of WT mice by ≈49%, whereas it completely abolished this response in arterioles of eNOS-KO mice. Simultaneous administration of INDO and L-NNA eliminated flow-induced responses in arterioles of WT mice. Dilations to carbaprostacyclin were similar at concentrations of 10⁻⁴ and 3×10⁻⁴ mol/L but decreased significantly at 10⁻³ mol/L in arterioles of eNOS-KO compared with those of WT mice. These findings demonstrate that, despite the lack of nitric oxidemediation, flow-induced dilation is close to normal in arterioles of eNOS-KO mice because of an enhanced release of endothelial dilator prostaglandins and suggest that this vascular adaptation may contribute to the regulation of peripheral resistance in eNOS-KO mice. (Circ Res. 1999;85:288-293.)

Key Words: transgenic mouse • intraluminal flow • endothelium • nitric oxide • prostacyclin

Considerable evidence demonstrates that endothelium-derived nitric oxide (NO) plays a critical role in the regulation of vascular tone.¹⁻³ One of the important mechanisms through which NO release is controlled is by alterations in wall shear stress during changes in blood flow.³⁴ Previous studies demonstrated that increases in perfusate flow elicit endothelium-dependent dilation of gracilus muscle arterioles, a response that is mediated by the combined release of NO and prostaglandins.⁵ It has also been demonstrated that reduced release of NO could lead to an enhanced arteriolar resistance, which may be involved in the pathogenesis of cardiovascular disorders, such as hypertension and atherosclerosis.⁶⁻⁷

The importance of the NO-related dilator mechanism is further underlined by studies showing a significant increase in blood pressure during systemic inhibition of nitric oxide synthase (NOS) with L-arginine analogs⁸⁻⁹ or when the gene encoding endothelial nitric oxide synthase (eNOS) is disrupted.¹⁰¹¹ Interestingly, however, there are quantitative differences in blood pressure in response to systemic administration of L-arginine analogs in wild-type (WT) mice compared with the level of blood pressure in eNOS knockout (KO) mice.¹² This led us to hypothesize that the cardiovascular system adapts to an acute inhibition of NO synthesis in a manner that is different from that observed with a chronic lack of NO. Thus, it seemed to be of interest to elucidate the possible changes or adaptation of flow-sensitive vasomotor mechanisms regulating the tone of arterioles in mice that have a targeted disruption of the gene encoding eNOS. It can be assumed that because of the absence of NO release from endothelial cells, flow-induced dilation is severely attenuated in arterioles of eNOS-KO mice, compared with the same response of skeletal muscle arterioles of WT control mice. Alternatively, it can be hypothesized that endothelial cells adapt to the chronic lack of NO synthesis and maintain a normal or close to normal regulation of vascular tone by upregulation of other mechanisms. To our knowledge, there have been no studies published that have examined the mechanisms involved in endothelial responses to changes in flow in skeletal muscle arterioles of either normal or eNOS-deficient mice.

Materials and Methods

Animals
Heterozygous eNOS (+/−) mice, originally developed by Shesely et al¹¹ were interbred to generate eNOS WT (+/+) and homozygous
mutant (−/−) mice. Mice were genotyped by Southern analysis of DNA as described previously11 with the eNOS WT used as littermate controls for the eNOS-KO mice. All protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College (NYMC) and conform to the current guidelines of the National Institutes of Health and the American Physiological Society for the use and care of laboratory animals. eNOS-KO and WT mice were bred in the Department of Comparative Medicine at NYMC. A total 28 male eNOS-KO mice and 22 male WT mice were used. Their average ages were 22.3±1.2 and 22.3±1.5 weeks, and their average body weights were 30.9±1.0 and 30.9±0.6 grams, respectively.

**Experimental Setup**

Experiments were conducted on isolated first-order gracilis muscle arterioles of male eNOS-KO and WT mice. Mice were killed by cervical dislocation. The dissection and isolation of vessels were similar as described earlier for rats.5 A segment, about 1 mm long, of an arteriole was isolated and cannulated with 2 glass pipettes in a vessel chamber (1 mL in volume) and suffused (1 mL/min) with physiological salt solution, buffered with NaHCO₃ (24.0 mmol/L) and 5% CO₂ plus ambient air to maintain the pH at 7.4. Intravascular pressure and temperature were maintained at 80 mm Hg and 37°C, respectively. Intraluminal flow was established by changing proximal and distal pressures, controlled by 2 pressure-servo systems (Living Systems Inc.). The efficacy of removal of the endothelium and the function of smooth muscle were assessed by loss of arteriolar diameter. In the presence of sodium nitroprusside (10⁻² mol/L, 3×10⁻³ mol/L, and 10⁻³ mol/L) was administered to the vessel chamber in a cumulative manner, and peak changes in diameter were recorded.

**Passive Diameter**

At the conclusion of each experiment, the suffusion solution was changed to a Ca²⁺-free solution containing 1 mmol/L EGTA. Vessels were incubated for 10 minutes to reach maximal diameter at 80 mm Hg perfusion pressure. The internal and external diameters of the arteriole were then measured to calculate wall thickness.

**Chemicals**

All chemicals were obtained from Sigma Chemical Co, except for L-NNA, which was purchased from Aldrich Chemical Co, and CP, which was obtained from Cayman Chemical. L-NNA (10⁻² mol/L) was dissolved in saline with sonication. INDO and CP were dissolved in DMSO (10⁻¹ mol/L and 10⁻² mol/L, respectively). Aliquots of CP were stored in ~20°C. All other solutions and drugs were prepared on the day of the experiments by dilution with the suffusion solution.

**Calculations and Statistics**

Passive diameter (internal) was used to assess the active (basal) tone generated by arterioles in response to intravascular pressure and to normalize the changes in diameter in response to various stimuli in each vessel to compare the results between groups of mice. Wall shear stress (WSS) was calculated as follows: WSS=4ηQ/r², where η is the viscosity of perfuse solution (~0.007 poise, at 37°C), Q is the perfuse flow, and r is the vessel radius. Wall thickness was calculated as the difference between external and internal radii.

Data are mean±SEM. The number of mice used for each experimental protocol is denoted by n. When 2 or more vessels were studied from a mouse, responses were averaged. Both absolute and normalized data were evaluated. Statistical significance was calculated by repeated-measures ANOVA followed by Tukey/Kramer multiple-comparison test. Student’s t test was also used, as appropriate. Significance level was taken at P<0.05.

**Results**

The characteristics of arterioles isolated from the gracilis muscle of WT and eNOS-KO mice are shown in the Table. Both basal and passive diameters were significantly smaller in arterioles of eNOS-KO mice compared with those of WT mice, whereas the basal tone, expressed as a percentage of passive diameter, and wall thickness of arterioles were not significantly different in eNOS-KO and WT mice.

In a total of 22 WT and 28 eNOS-KO mice, changes in diameter of arterioles in response to step increases in intraluminal flow were examined. Increasing flow from 0 to 10 μL/min elicited significant increases in arteriolar diameter in both WT and eNOS-KO mice (Figure 1, top). The maximal changes in diameter in response to increases in perfuse flow

**Characteristics of Gracilis Muscle Arterioles of Mice**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>WT</th>
<th>eNOS-KO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diameter, μm</td>
<td>87.7±3.1</td>
<td>80.5±2.5*</td>
</tr>
<tr>
<td>Passive diameter, μm</td>
<td>137.6±3.2</td>
<td>122.9±3.6*</td>
</tr>
<tr>
<td>Wall thickness, μm</td>
<td>21.3±1.0</td>
<td>19.6±1.1</td>
</tr>
<tr>
<td>Basal tone (% of passive diameter)</td>
<td>63.6±1.5</td>
<td>65.6±0.9</td>
</tr>
</tbody>
</table>

*Significant difference compared with WT mice.

Data are mean±SEM. All measurements were made at 80 mm Hg of intravascular pressure. Passive diameter and wall thickness were measured in a Ca²⁺-free solution. Basal diameter was obtained before the onset of flow (n=22 and n=28 in WT and eNOS-KO mice, respectively).

**Carbaprostacyclin-Induced Dilation**

In separate experiments, arteriolar responses to carbaprostacyclin (CP), a stable analog of prostacyclin, were determined at 80 mm Hg pressure without intraluminal flow in the 2 groups of mice. CP (10⁻² mol/L, 3×10⁻³ mol/L, and 10⁻³ mol/L) was administered to the vessel chamber in a cumulative manner, and peak changes in diameter were recorded.
were similar (21.9±1.6 and 20.1±1.2 μm at 10 μL/min, respectively). That the dilation of arterioles from eNOS-KO and WT mice is nearly identical can also be seen in the middle panel of Figure 1, in which the normalized diameter as a function of perfusate flow is depicted. Increases in flow elicited comparable increases in calculated WSS in the 2 types of vessels (Figure 1, bottom).

Although removal of the endothelium did not affect basal diameters, it completely eliminated flow-induced dilation in vessels of both WT and eNOS-KO mice (Figure 2, top and bottom, respectively), indicating that endothelial factors are responsible for the mediation of this response. In separate experiments, the endothelial mediators contributing to flow-induced dilation of arterioles were investigated. The role of prostaglandins in mediation of flow-induced dilation of arterioles were investigated. The role of prostaglandins in mediation of flow-induced dilation of arterioles was examined by comparing the responses before and after administration of INDO. Basal arteriolar diameters were not significantly affected by INDO, which, however, significantly inhibited flow-induced dilation by 49.4±10.4% (P<0.05) in arterioles of WT mice (Figure 3, top). In contrast, INDO abolished flow-dependent dilation in vessels of eNOS-KO mice (Figure 3, bottom). In a separate group of experiments, the role of NO in arteriolar responses to increases in flow was assessed by using L-NNA. L-NNA did not significantly affect basal arteriolar diameter but inhibited...
flow-induced dilation by 51.0 ± 3.6% (P < 0.05) in arterioles of WT mice (Figure 4, top), whereas it had no effect on the response in arterioles of eNOS-KO mice (Figure 4, bottom). Additional administration of INDO completely eliminated flow-induced dilation in arterioles of both WT and eNOS-KO mice (Figure 4, top and bottom, respectively). These findings demonstrate that both NO and prostaglandins mediate flow-induced dilation in gracilis muscle arterioles of WT mice, whereas prostaglandins alone contribute to the same degree of dilation in arterioles of eNOS-deficient mice.

To characterize the reactivity of vascular smooth muscle to prostaglandins, arteriolar responses to CP, a stable analog of prostacyclin, were assessed. Figure 5 shows that there was no significant difference in vasodilator responses to the lower concentrations of CP (10⁻⁸ mol/L and 3×10⁻⁹ mol/L), but there was a significant reduction in the response to the higher concentration of CP (10⁻⁷ mol/L) in arterioles of eNOS-KO compared with those of WT mice.

**Discussion**

The new findings of the present study are as follows: (1) in male eNOS-KO mice, dilation of isolated skeletal muscle arterioles to flow/shear stress is close to normal, because of upregulation of endothelial prostanoid-mediated responses, and (2) in arterioles of WT mice, corelease of NO and prostaglandins mediates flow-induced dilation. Endothelium-derived NO mediates dilation in response to physical stimuli, eg, shear stress, and to vasoactive agents, eg, acetylcholine, bradykinin, and substance P, in conduit vessels as well as microvessels. In vivo, the primary stimulus for the release of NO from the vascular endothelium is an increase in shear stress, a mechanism that has been shown to contribute importantly to the regulation of organ blood flow and peripheral resistance. Our previous studies showed that in arterioles of spontaneously hypertensive rats, endothelial NO release is reduced in response to shear stress, resulting in an impaired flow-dependent dilation, suggesting further that a strong association exists between NO release and peripheral resistance. After exercise training, on the other hand, the endothelial synthesis of NO in arterioles of rats and NOS gene expression in aorta of dogs is enhanced, eliciting greater arteriolar dilation to increases in shear stress.

Given that NO plays an important role in the mediation of shear stress–induced dilation, it was logical to hypothesize that in the absence of eNOS, arteriolar dilation to increases in shear stress is greatly diminished. However, the greater increase in blood pressure of WT mice administered L-NNA acutely, compared with the level of blood pressure found in eNOS-KO mice, led us to speculate that, perhaps as a result of an adaptive compensatory mechanism, flow-induced dilation is still maintained close to normal in vessels of eNOS-KO mice. To test this hypothesis, flow-induced dilation and the role of endothelial factors mediating this response were investigated in gracilis muscle arterioles of eNOS-KO and WT mice.

The average age and body weight of the 2 strains of mice were similar, and the vessels of the mice were carefully selected with respect to their anatomic location and branch order. Yet, the basal diameter and maximal passive diameter of gracilis arterioles of eNOS-KO mice were smaller than those of WT mice. The reason for this difference is not known, but it is likely to be related to the chronic lack of endothelial production of NO. In hypertensive rats, an experimental model associated with reduced endothelial synthesis of NO, the diameter of microvessels is reduced, a finding that is suggestive of an important role for NO in vascular remodeling. In the present study, there were no differences noted in the wall thickness of arterioles of the 2 strains of mice. Previous studies also showed no differences in lumen diameter and wall thickness of carotid and femoral arteries of eNOS-KO mice compared with those of WT mice. Interestingly, however, in small mesenteric arteries of

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** Changes in diameter of gracilis muscle arterioles of WT (top) and eNOS-KO (bottom) mice as a function of perfusate flow in control conditions, in the presence of L-NNA 10⁻⁴ mol/L or in the presence of L-NNA plus INDO 10⁻⁵ mol/L (L-NNA + INDO). *Significant difference between 2 adjacent curves as indicated by ANOVA. #Significant difference between control or in the presence of L-NNA and in the presence of L-NNA + INDO as indicated by ANOVA.

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Changes in diameter, expressed as a percentage of passive diameter (PD), of gracilis muscle arterioles from WT and eNOS-KO mice in response to CP. *Significant difference vs WT mice.
eNOS-KO mice, an increased wall thickness was observed, an alteration that may favor an increase in peripheral resistance.

In the present study, the basal myogenic tone of vessels in the 2 strains of mice was not significantly different, indicating a similar responsiveness to intraluminal pressure. This is of importance, given that the level of myogenic tone can influence the magnitude of flow/shear stress–dependent dilation. In response to increases in intraluminal flow, arterioles of both strains of mice exhibited substantial dilations. The actual changes in diameter, the normalized dilator responses, and the level of shear stress were similar in arterioles of WT and eNOS-KO mice (Figure 1). These findings suggest that despite the absence of NO synthesis in the endothelium of arterioles of eNOS-KO mice, dilation of arterioles to increases in perfusate flow/shear stress is preserved. We have also found that the flow-dependent dilation of arterioles was entirely due to endothelial factors, because removal of endothelium eliminated the response in vessels of both groups of mice (Figure 2). To study the endothelial mediation of responses, inhibitors, which interfere with the synthesis of NO and prostaglandins, were used. In the absence of perfusate flow, the basal diameter of arterioles was not affected by $10^{-4}$ mol/L L-NNA or $10^{-5}$ mol/L INDO, inhibitor concentrations that were shown to block completely the release of NO or prostaglandins, respectively, in gracilis muscle arterioles of the rat. Similar to these results, previous studies found only a minimal basal release of NO in aorta and pulmonary artery of eNOS knockout mice.

As for the endothelial factors responsible for the mediation of flow-induced responses in gracilis muscle arterioles of WT mice, at 80 mm Hg of perfusion pressure, inhibition of either NO or prostaglandin synthesis alone significantly reduced arteriolar dilation to increases in perfusate flow by $\approx 50\%$ (Figures 3 and 4, top). Moreover, inhibition of the synthesis of both mediators eliminated flow-induced dilation (Figure 4, top). These findings correspond to our previous report, namely, that NO and prostaglandins are coreleased in rat gracilis muscle arterioles in response to increases in flow and are responsible for the ensuing vasodilatation. In contrast, in arterioles of eNOS-deficient mice, L-NNA had no effect, whereas additional INDO completely inhibited the flow-dependent dilation (Figures 3 and 4, bottom). Because previous studies suggested that the presence of NO may interfere with the synthesis of prostaglandins, one could surmise that a chronic lack of NO in eNOS-KO mice may have elicited changes in the function of the endothelium. That INDO alone completely eliminated flow-induced dilation indicates that this response is solely mediated by dilator prostaglandins. The results also suggest that in the absence of mediation by endothelial NO, an increased contribution of prostaglandins is responsible for the close to normal flow-induced dilation in arterioles of eNOS-KO mice.

Because previous studies indicated that NO donor–induced relaxations are reduced when eNOS is overexpressed but are, conversely, potentiated in eNOS-deficient mice, we aimed to elucidate whether an enhanced sensitivity to or an enhanced release of endothelial prostaglandins in response to shear stress is responsible for the adaptation of arterioles in mice deficient in the eNOS gene. To this end, arteriolar responses of the 2 strains of mice to a stable prostacyclin analog, CP, were contrasted. The results show that at lower concentrations, CP elicited similar dilations in vessels of the 2 groups of mice, whereas at higher concentrations, responses of arterioles of eNOS-deficient mice were reduced compared with those of WT mice (Figure 5). The reason for the reduced sensitivity of arterioles to CP is not clear, but the results, nevertheless, exclude the possibility that an enhanced sensitivity of arteriolar smooth muscle to vasodilator prostaglandins could account for the compensation and suggest that an enhanced release of prostaglandins is responsible for the maintenance of flow-dependent response in eNOS-KO mice.

There are other published studies that suggest a physiological adaptation of the vasculature to the absence of the eNOS gene. A recent study showed similar reactive hyperemic responses of coronary vessels of eNOS-KO and WT mice. In this context, it has also been shown that the production of endothelium-derived hyperpolarizing factor and prostaglandins is enhanced in vessels of mice deficient in the eNOS gene. During a chronic absence of NO, these mediators may act as a protective mechanism in the maintenance of endothelial function, as has been documented in the mesenteric artery and coronary artery of eNOS-KO mice, as well as coronary vessels of dogs and carotid artery of rabbits.

A possible underlying mechanism for the enhanced release of prostaglandins observed in the present study is suggested by previous reports showing an upregulation of the cyclooxygenase type I isofrom (COX 1) in the endothelium of dog coronary artery and an overproduction of vasodilator prostaglandins in mesenteric arteries of rats, resulting in part from COX-2 expression, after chronic inhibition of eNOS. It is also possible that the intracellular mechanisms responsible for the compensation for the lack of NO in eNOS-deficient mice are vascular bed specific. For example, in gracilis muscle arterioles of male WT mice, the dilation to acetylcholine is mediated mainly by endothelium-derived hyperpolarizing factor. Responses to acetylcholine are mediated by neuronal NO in cerebral and prostaglandins in mesenteric arteries, and other, as yet unidentified, mediators in coronary artery of eNOS-KO mice. Thus, it is tempting to speculate that perhaps one or more redundant compensatory mechanisms become activated after selective gene deletion. Nevertheless, the investigation of these adaptive mechanisms may help to better understand the interaction among the various endothelium-derived vasoactive mediators to maintain normal vascular function.

In conclusion, the present study is the first to demonstrate that flow-induced dilation is mediated by both endothelial NO and prostaglandins in skeletal muscle arterioles of normal (WT) mice, whereas it is mediated exclusively by prostaglandins in those of eNOS-KO mice. This alteration may play a crucial role in the maintenance of shear stress–sensitive mechanisms in skeletal muscle arterioles and the regulation of peripheral resistance in eNOS-deficient mice.
Acknowledgments
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