Estrogen Preserves Regulation of Shear Stress by Nitric Oxide in Arterioles of Female Hypertensive Rats

An Huang, Dong Sun, Gabor Kaley, Akos Koller

Abstract—Previously we found that flow-induced arteriolar dilation in male spontaneously hypertensive rats (SHR) is significantly impaired, due to the absence of the nitric oxide (NO)-mediated portion of the response, resulting in an elevation of maintained wall shear stress. Since estrogen has been shown to affect NO-mediated responses, we hypothesized that in female SHR (fSHR) the NO-mediated portion of flow-induced responses is preserved. Gracilis muscle arterioles (~45 to 55 μm) from 12-week-old fSHR, ovariectomized fSHR (OV fSHR), or ovariectomized and supplemented with estrogen fSHR (OVE fSHR) were isolated, cannulated, and pressurized at 80 mm Hg of perfusion pressure. Arteriolar dilations elicited by step increases in perfusate flow from 0 to 25 μL/min were significantly less (by ~30%) in OV fSHR compared with fSHR and OVE fSHR (Δ19 ± 1.5 versus 26.0 ± 0.9 and 26.8 ± 2.0 μm, respectively at maximum flow rate). Inhibition of prostaglandin synthesis with indomethacin elicited a ~50% reduction in flow-dependent dilation in all three groups of rats. Nω-nitro-L-arginine (L-NNA) significantly inhibited flow-induced responses in arterioles of fSHR and OVE fSHR (by ~50%) but not in those of OV fSHR. Constrictions to norepinephrine (10^{-7} - 3×10^{-7} mol/L) were significantly greater (up to ~40%) in arterioles of OV fSHR compared with those of fSHR and OVE fSHR. These differences, however, were abolished in the presence of L-NNA. In conclusion, estrogen seems to preserve the NO-mediated portion of flow/shear stress-induced dilation in female hypertensive rats resulting in a lower maintained wall shear stress in female than in male SHR. The lower wall shear stress may contribute to the mechanisms by which estrogen lowers systemic blood pressure and the incidence of cardiovascular diseases in women (Hypertension. 1998;31[part 2]:309-314.)

Key Words: female spontaneously hypertensive rats • estrogen • arterioles • nitric oxide • wall shear stress

The prevalence of essential hypertension is lower in premenopausal women than in age-matched men. Also, hypertension develops more rapidly and becomes more severe in male than in female animals in genetic and other hypertensive models. These findings suggest that female hormones may delay the increase in peripheral resistance associated with hypertension.

Several studies have already described the beneficial effect of estrogen on the cardiovascular system, yet the underlying mechanisms of these effects are not fully understood. In addition to humoral and neural factors, regulation of arteriolar resistance by local factors, intrinsic to the vascular wall are important in the development of peripheral vascular resistance. Studies investigating the role of vascular factors showed that the chronic presence of estrogen in female animals reduces the tone of aortic smooth muscle. In addition to the possible direct effect of estrogen on smooth muscle, it was shown that estrogen potentiates the endothelial function of vessels of normotensive as well as hypertensive animals, and elicits increases in plasma levels of nitric oxide and nitrate. It has also been demonstrated that estrogen upregulates NO synthase in vascular tissue.

All of the aforementioned studies, however, were done in large conduit vessels or under conditions in which NO synthase was stimulated by agonists (such as acetylcholine or substance P), which are unlikely to be involved in the in vivo regulation of arteriolar resistance. More recent studies demonstrated, however, that in postmenopausal women estrogen reduced blood pressure and enhanced basal but not acetylcholine-induced NO release in forearm resistance arteries.

One of the primary local factors that determines the resistance of arterioles is the myogenic mechanism eliciting constriction to increases in intraluminal pressure. Thus, modulation of the strength of myogenic constriction by dilator factors could be important in the development of peripheral resistance. Indeed, we have found previously that pressure-induced myogenic constriction is modulated by the basal and shear stress-induced release of NO. The essential role for NO, released from endothelium of resistance vessels, is shown by studies demonstrating that systemic application of inhibitors of NO synthase elicits great increases in blood pressure.

In subsequent studies we found that the basal tone is weaker in arterioles of female compared with male rats, and that this is due to a greater basal release of NO, stimulated by the long-term presence of estrogen in female rats or in ovariectomized female rats supplemented with estrogen.

It was also shown that myogenic constriction is enhanced and that dilations to agonists and flow/shear stress are...
Impaired in arterioles of male hypertensive rats primarily due to absence of NO release. In contrast, in female hypertensive rats, we found that release of NO to agonists and modulation of myogenic tone by basally released NO are still present in skeletal muscle arterioles, but only if estrogen is present. In vivo, one of the primary stimuli for the release of NO, which then can affect peripheral resistance, is the continuous stimulation of endothelium by blood flow/wall shear stress.

Thus, the aim of our study was to investigate the magnitude and mediation of shear stress-induced dilation in skeletal muscle arterioles of female hypertensive rats. Based on the aforementioned studies, we hypothesized that arteriolar dilation to flow/shear stress mediated by NO is still present in skeletal muscle arterioles of female SHR.

**Methods**

**Experimental Animals**

Twelve-week-old female SHR (Charles River Laboratory Wilmington, Mass.) were divided into three groups: normal females (fSHR), ovariectomized females (OV fSHR), and ovariectomized females with estrogen replacement (OVE fSHR). Ovariectomy was performed at 9 weeks of age under methoxyflurane (Mefofoxane anesthesia. One week after the operation, the rats were divided into two groups. One group received injections of 17β-estradiol benzoate (50 μg/kg, every 3–4 days) subcutaneously for 24 hours. The other rats were used as controls. After 48 hours, the animals were killed by an overdose of pentobarbital sodium injected into the abdominal aorta with a 10-mL syringe containing 0.1 mL of heparin (1000 IU/mL). The blood samples were centrifuged immediately (3000 rpm at 4°C for 30 minutes) to obtain the plasma. The plasma was kept at -80°C for measurement of plasma estradiol concentration.

**Perfusion System**

As described previously, a pair of glass micropipettes (both proximal [inflow] and distal [outflow]) in the vessel chamber were connected with silicone tubing to a pressure-servo syringe system (Living Systems Inc). Perfusion flow was established at a constant intravascular pressure by changing proximal and distal pressures to an equal level but in opposite directions to keep midpoint luminal pressure constant. The flow was measured by a ball flowmeter (FL-300, Omega) calibrated by a Harvard perfusion pump in a flow range of 0 to 100 μL/min.

**Experimental Procedure**

The changes in diameter of arterioles in response to increases in perfusate flow were assessed in control and various conditions. The vessels were equilibrated at 80 mm Hg of perfusion pressure for 1 hour, in a no-flow condition, to develop spontaneous tone, then perfusate flow was increased from 0 to 25 μL/min in 5-μL/min steps. Each flow step was maintained for 5 minutes to allow the vessels to reach stable diameter. After the flow-diameter relation was obtained, flow was stopped, and then approximately 70 minutes later, responses of arterioles to various doses of NE (10−2, 2×10−2, and 3×10−2 mol/L) were tested. In the first experimental series, the role of prostaglandins in the flow-induced responses was assessed by the use of indomethacin (10−2 mol/L), an inhibitor of cyclooxygenase. After a control flow-diameter curve was obtained, the vessel was incubated with indomethacin for 30 minutes. Then, changes in diameter in response to increases in perfusate flow were reassessed. In the second series of experiments, after a control flow-diameter curve was obtained, the vessels were subjected to L-NNA (10−4 mol/L), an inhibitor of NO synthase. After a 20-minute incubation period, the flow-diameter relation and arteriolar responses to NE were once more assessed. In these experiments, after L-NNA, indomethacin was also administered, and the flow-diameter curve was again determined.

Responses to vasoactive agents were tested at 80 mm Hg perfusion pressure in no-flow conditions. Agonists were added to the reservoir connected to the vessel chamber, and final concentrations were reported. To assess the active tone generated by the arterioles in response to intravascular pressure, at the conclusion of each experiment, the suffusion solution was changed to a Ca2+-free PS2 solution containing sodium nitroprusside (10−4 mol/L) and EGT (10−2 mol/L). The vessels were incubated for 10 minutes, and the passive diameter of arterioles at 80 mm Hg perfusion pressure was measured.

**Isolation of Arterioles**

Experiments were conducted on isolated second-order arterioles (~50 μm in internal diameter) of gracilis muscle. Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (Nembutal sodium, 50 mg/kg). The isolation procedure of arterioles has been described previously. Briefly, the muscle was excised and placed into a refrigerated dissecting dish containing a cold (0 to 4°C) modified physiological salt (PS1) solution for preparation. The PS1 solution contained (in mmol/L): 145 NaCl, 5 KCl, 2.5 CaCl2, 0.5 MgSO4, 10 NaH2PO4, 5.0 dextrose, 2 pyruvate, 0.02 EDTA, and 3.0 3-(N-morpholino)-propanesulfonic acid to reach a pH of 7.4. The muscle was placed open as a flat sheet of tissue and pinned to the bottom of the silicone-lined base of a Petri dish. Rats were killed by an overdose of pentobarbital sodium injected into the abdominal aorta.

With microsurgery instruments and an operating microscope (Leica), a segment (~1 mm long) of an arteriole was isolated from gracilis muscle and surrounding tissue and transferred to the vessel chamber. The chamber contained a pair of glass micropipettes filled with a second physiological salt (PS2) solution at room temperature. The PS2 solution was made by mixing a gas mixture of 21% O2/5% CO2 balanced with N2 at pH 7.4 (37°C). From a reservoir, the vessel chamber was continuously supplied with PS2 solution at a rate of 40 μL/min. After the vessel was mounted on the proximal pipette and secured with sutures, the perfusion pressure was raised to 20 mm Hg to clear the blood from the lumen, and then the other end of the vessel was mounted on the distal pipette. To flush the vessel and cannula, the system was perfused for several minutes.

**Statistics**

The data are presented as mean ± SE. Only one vessel was studied from each animal. Flow-induced responses were expressed as change in diameter at each flow step. Wall shear stress was calculated after vessel...
diameter reached a steady state value by the following equation:
\[ \frac{4\eta Q}{r} \text{, where } \eta \text{ is the viscosity of the perfusate (0.007 poise at 37°C), } Q \text{ is the perfusate flow, and } r \text{ is the vessel radius.} \]
Response to vasoactive agents were expressed by change in diameter, as the percentage of basal diameter. Statistical analyses were done by two-way ANOVA, followed by Tukey's posthoc test, and paired and grouped Student's t tests, as appropriate. A value of \( P < 0.05 \) was considered significant.

### Results

The systolic blood pressure, heart rate, and plasma 17β-estradiol concentration are shown in Table 1. The increase in blood pressure and decrease in heart rate after ovariectomy of SHR did not reach a level of significance. Plasma 17β-estradiol concentration however, was significantly reduced after ovariectomy and was normalized by estrogen replacement. Table 1 also shows the changes in body weight, uterus weight, and the ratio of uterus weight/body weight in SHRs, OV SHRs, and OVE SHRs. The uterus weight of OV SHRs was significantly lower than that of ISHR or OVE SHRs (\( P < 0.01 \)). In contrast, the body weight of OV SHRs was significantly greater than that of the other two groups (\( P < 0.05 \)). As a result, the ratio of uterus weight/body weight was significantly less in OV SHRs compared with the other two groups (\( P < 0.01 \)).

The active diameters of arterioles, obtained in response to an elevation of intravascular pressure to 80 mm Hg in the absence of perfusate flow, are summarized in Table 2. The basal diameter was significantly smaller in OV ISHR than in the other groups. In the same conditions, but in Ca²⁺-free solution, the passive diameter of each group was obtained and was found to be similar (~100 μm). Expressing the active diameter as a percentage of the corresponding passive diameter indicates a significantly greater basal tone in arterioles of OV ISHR than in ISHR and OVE ISHR.

Fig 1 summarizes the changes in diameter of arterioles of three groups of female rats in response to step increases in perfusate flow. The change in diameter in response to increases in flow was significantly greater in arterioles of ISHR and OVE ISHR than in those of OV ISHR at each flow step. For example, at 25 μL/mm flow, the arteriolar diameters of ISHR and OVE ISHR were ~20% greater than those of OV ISHR. For comparison, flow-dependent dilations of gracilis arterioles of male SHR, obtained previously,22 are also included in Fig 1. The results indicate that flow-induced dilation is greater in arterioles of ISHR and OVE ISHR than those of OV ISHR and male SHR, thus estrogen accounts for the greater flow-induced dilation in arterioles of ISHR and OVE ISHR.

From the flow and diameter data obtained, wall shear stress was calculated and plotted against the changes in arteriolar diameter. Fig 2 shows that a given step increase in wall shear stress elicits a significantly greater increase in diameter of arterioles of ISHR and OVE ISHR compared with those of OV ISHR, resulting in a significant leftward shift of the shear stress–diameter curves of ISHR and OVE ISHR. The plot reveals that the maintained shear stress value is ~100 dyn/cm² in OV ISHR and ~50 dyn/cm² in both ISHR and OVE ISHR. Again, the shear stress–diameter curve of arterioles of OV ISHR overlaps the curve obtained in vessels of male SHR.25

Next we investigated the factors responsible for the flow-induced dilation of arterioles of ISHR with or without estrogens. First we examined the role of prostaglandins by investigating the flow–diameter relation in the presence of indomethacin. We found that indomethacin alone did not significantly affect basal diameter (Table 2) but significantly reduced the arteriolar dilation to increases in perfusate flow in all groups of rats (Fig 3). In all groups of ISHR, the reduction of the maximum response was similar (between 50% and 60%).

After control responses were obtained, L-NNA was used to examine the involvement of NO in flow-induced responses (Fig 4). L-NNA abolished the difference in basal tone in three groups of ISHR (Table 2) and significantly (by ~50%) shifted down the flow–diameter curves in both ISHR (top) and OVE ISHR (bottom). In arterioles of OV ISHR (middle), however, the inhibition was not significant. These findings indicate that L-NNA has a significantly greater effect on flow-induced responses in arterioles of ISHR and OVE ISHR than in those of OV ISHR. In the presence of L-NNA, administration of

### Table 1. Effects of Ovariectomy and Estrogen Replacement on Blood Pressure, Heart Rate, Plasma Estradiol Concentration, Body Weight, Uterus Weight, and Uterus Weight/Body Weight Ratio

<table>
<thead>
<tr>
<th>Measure</th>
<th>ISHR</th>
<th>OV ISHR</th>
<th>OVE ISHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP, mm Hg</td>
<td>181±3</td>
<td>206.6±10.3</td>
<td>195.2±6.8</td>
</tr>
<tr>
<td>HR, mm⁻¹</td>
<td>499.8±12.0</td>
<td>444.2±7.6</td>
<td>494.8±12.1</td>
</tr>
<tr>
<td>F, pg/ml</td>
<td>125.8±3.4</td>
<td>3.0±0.9</td>
<td>144.5±2.8</td>
</tr>
<tr>
<td>RW, g</td>
<td>174.3±2.0</td>
<td>202.6±6.6</td>
<td>150.0±2.2</td>
</tr>
<tr>
<td>UW, g</td>
<td>0.319±0.03</td>
<td>0.684±0.004</td>
<td>0.372±0.2</td>
</tr>
<tr>
<td>UW/RW</td>
<td>0.183±0.02</td>
<td>0.407±0.002</td>
<td>0.237±0.01</td>
</tr>
</tbody>
</table>

BP indicates systolic blood pressure, HR, heart rate, E, estrogen, BW, body weight, UW, uterus weight.

### Table 2. Effects of L-NNA and Indomethacin on Basal Arteriolar Diameter of ISHR

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ISHR</th>
<th>OV ISHR</th>
<th>OVE ISHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>AD, μm</td>
<td>51±1.7</td>
<td>47.9±2.9</td>
</tr>
<tr>
<td></td>
<td>PD, μm</td>
<td>987.2±29</td>
<td>1039.3±15</td>
</tr>
<tr>
<td></td>
<td>AD/PD, %</td>
<td>57.9±0.8</td>
<td>45.9±1.5</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>AD/PD, %</td>
<td>56.7±2.5</td>
<td>41.0±2.2</td>
</tr>
<tr>
<td>L-NNA</td>
<td>AD/PD, %</td>
<td>46.0±15†</td>
<td>43.0±2.3</td>
</tr>
<tr>
<td>Indomethacin+L-NNA</td>
<td>AD/PD, %</td>
<td>42.3±14†</td>
<td>39.6±17†</td>
</tr>
</tbody>
</table>

AD, active diameter; PD, passive diameter.
†Significant difference from other groups.
*Significant difference from control.
indomethacin practically eliminated the arteriolar dilation to increases in perfusate flow in all groups.

Changes in normalized diameter of arterioles in responses to NE are shown in Fig 5 Increasing dosages of NE elicited significantly greater constrictions of arterioles from OV fSHR than those from fSHR and OVE fSHR (top) These differences were, however, abolished after inhibition of NO synthase with L-NNA (bottom).

**Discussion**

The new findings of this study are (1) that wall shear stress is maintained at a significantly lower range in skeletal muscle arterioles of female SHR compared with male SHR, (2) that this is due to the still-present NO mediation of the flow/shear stress-induced dilation in female SHR, (3) that this response is dependent on the chronic presence of estrogen in female rats, and (4) that arteriolar constrictions to NE are significantly reduced by the concomitant release of NO but only if estrogen is present in female hypertensive rats.

Hypertension is known to be associated with an elevated peripheral resistance for which all the causes are not yet fully elucidated. Interestingly, population-based human studies showed that women have a lower prevalence of and less severe hypertension before menopause leading to the hypothesis that female hormones have significant roles in the long-term regulation of peripheral resistance. It is believed that in addition to neural and humoral mechanisms local factors intrinsic to the wall of the vessels are important contributors to the regulation of peripheral resistance. These factors are related to the function of endothelium and arteriolar smooth muscle.

Previous studies of large isolated conduit vessels suggest that release of endothelial NO is greater in females than in males and that ovariectomy eliminated, whereas supplemental estrogen therapy restored, this difference. Because these vessels do not contribute significantly to the peripheral resistance, it was of interest to elucidate the possible role of estrogen in the long-term regulation of peripheral resistance. In isolated arterioles of skeletal muscle of normotensive female rats, we found a greater dilation to agonists known to release NO than in those of normotensive males. In addition, it was shown that the greater basal release of NO causes a significantly greater reduction in the magnitude of myogenic constriction in arterioles isolated from female rats compared with those of male rats. These findings have obvious physiological importance, because arterioles are the vessels primarily responsible for the development of peripheral resistance. Furthermore, it was also shown that dilations to NO-dependent agonists (e.g., substance P) are also greater in arterioles of female compared with those of male hypertensive rats. Dilations in the NO donor sodium nitroprusside, however, were similar in both normotensive and hypertensive rats of both sexes, indicating that neither hypertension nor estrogen have substantial effect on the responsivity of arteriolar smooth muscle to NO.
Previous studies showed that constriction to phenylephrine is reduced in mesenteric arterioles of female rats compared with those of female rats after ovariectomy and that the difference is due to a greater release of NO in the vessels of the non-ovariectomized females. These changes were also accompanied by corresponding changes in systemic blood pressure. In the present study we found that NE-induced constrictions in female arterioles are weaker if estrogen is present and that this is due to the greater concomitant release of NO. Because in hypertension it is likely that arterioles are exposed to a higher level of circulating and/or neurally released NE, the greater release of NO in female hypertensive rats can have a greater counteractive effect on the constriction elicited by NE. Thus, NE may have a significantly greater effect on peripheral resistance and blood pressure in male hypertensive subjects as well as in female subjects after menopause, in whom estrogen levels are decreased.

Regulation of peripheral resistance is influenced by the tone of skeletal muscle arterioles, which is regulated by several local and remote mechanisms. Previous studies showed that alterations in the function of the local factors can contribute to the increase in peripheral resistance in hypertension. Several pieces of evidence suggest that endothelium derived NO is one of the primary factors able to counteract pressure-, NE-, angiotensin-, and endothelin-induced constrictions, responses that are enhanced in hypertension. Indeed, systemic inhibition of NO synthase elicits great increases in systemic blood pressure. In vivo, one of the primary stimuli for NO release is an increase in wall shear stress during increases in blood flow velocity or viscosity. Previously we found that flow-dependent dilation and, thus, regulation of wall shear stress, is altered in male SHRs, resulting in an elevation of wall shear stress in skeletal muscle arterioles. This increase in wall shear stress is caused by the impairment of the NO-mediated portion of shear stress-induced dilation. Thus, the aim of the present studies was to elucidate whether the NO-mediated portion of flow-dependent dilation is still present in arterioles of female hypertensive rats.

Comparison of the findings of the present and previous studies revealed that flow-dependent dilation is greater in female than in male SHRs. That the greater response is dependent on a greater release of NO and the presence of estrogen in female SHRs is indicated by the fact that the difference disappears after inhibition of NO synthesis or if female rats are ovariectomized. In contrast, if ovariectomized SHRs received estrogen, the greater flow-dependent arteriolar response is restored. It is also of note that flow-induced dilation in arterioles is completely blocked by removal of the endothelial cell layer. Thus, the presence of estrogen in female hypertensive rats maintains the endothelial synthesis of NO in skeletal muscle arterioles, manifested by a substantial shear stress-induced release of NO.

We speculate that the physiological importance of our findings is that the greater release of NO in arterioles of SHRs to flow/shear stress tends to reduce peripheral resistance by eliciting a greater suppression of the constrictor mechanisms, compared with what occurs in vessels of male SHRs. Together with the augmented concomitant release of NO in response to NE, the greater NO release in response to shear stress may delay the manifestation or development of hypertension that other-
wise is likely to be genetically determined in a similar manner in male and female SHR. This speculation is further supported by the finding that the maintained arteriolar wall shear stress is lower in fSHR than in ovariectomized fSHR and male SHR, suggesting that in the presence of estrogen the greater NO synthesis elicits a lower power dissipation in the female circulatory system by increasing arteriolar diameter. Thus, a lower systemic blood pressure is required to provide for an adequate cardiac output and blood flow to tissues in female hypertensive rats. Indeed, previous studies showed that estrogen augments the contribution of NO to regulate blood pressure in transgenic hypertensive rats expressing the mouse Ren-2 gene and that estrogen attenuates the development of hypertension in SHRs.

In conclusion, we demonstrate for the first time that both basal and shear stress-stimulated release of NO is greater in arterioles of female hypertensive rats than in males or ovariectomized females. This results in a reduced arteriolar wall shear stress, which may contribute to the delay in the increase in peripheral resistance and systemic blood pressure in female compared with male genetically hypertensive rats. Similar mechanisms may operate in humans as well.

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