Laboratory Studies

Crucial Role of Endothelium in the Vasodilator Response to Increased Flow in Vivo

ULRICH POHL, JÜRGEN HOLTZ, RUDI BUSSE, AND EBERHARD BASSENGE

SUMMARY Experiments were designed to investigate the importance of vascular endothelium in the vasomotor response to increases in flow as observed in conduit arteries (flow-dependent dilation). The functional integrity of the endothelial cells underneath the diameter sensors was tested by intrarterial acetylcholine (local acetylcholine dilation) applied proximally to the sensors while a constant flow was maintained. Unilateral augmentation of femoral arterial flow (4.6 ± 1.9-fold) induced by peripheral vasodilation or by arteriovenous shunt, elicited dilation (increase in diameter, 116 ± 91 μm) in 18 of 23 dogs, whereas the diameter of the contralateral control artery was not affected. Mechanical removal of the endothelial cells by means of a balloon catheter abolished both the flow-dependent dilation and the local acetylcholine dilation, whereas the vasomotor responses to norepinephrine and nitroglycerin were not affected. Brief perfusions (1 minute) of the arteries with cell-free hydrogen peroxide solution (90 mM) also abolished the flow-dependent dilation and attenuated the local acetylcholine dilation (by 27 ± 19%; \( p < 0.02 \)), while the responses to norepinephrine and nitroglycerin were not altered. These results suggest that endothelial cells act as mediators of flow-dependent dilation. (Hypertension 8: 37-44, 1986)

KEY WORDS • flow-dependent dilation • vascular endothelium • endothelium-mediated dilation • hydrogen peroxide

THE dilator response of conduit arteries to an augmentation of blood flow (flow-dependent dilation) was observed more than 50 years ago,1 and flow-dependent dilation has been suggested as the principal response in a variety of physiological vascular adaptations, such as collateralization and long-term diameter adaptation to increased flow loads.2 However, the underlying mechanism of this vasodilation is still a matter of debate. Originally the vasodilation was thought to be elicited by a peripheral conducting mechanism,1,3 but more recent experiments clearly indicate that a local mechanism is responsible.4 This explanation implies that vascular wall structures sensitive to flow changes are involved.

In view of the accumulating evidence for the modifying role of the vascular endothelium on vascular reactivity, and based on preliminary experiments in isolated arteries, we proposed that the vasodilation in response to flow increases is an endothelium-mediated reaction.5 The goal of the present study was to demonstrate that the endothelium is functionally important in flow-dependent dilation under in vivo conditions.

Materials and Methods

Experiments were performed in 23 mongrel dogs (estimated age, 1–5 years; weight, 25–39 kg) of either sex. The animals were anesthetized with sodium pentobarbital (Nembutal), 25 to 35 mg/kg i.v. and ventilated with room air. Additional doses of pentobarbital were injected throughout the experiment.

Vasodilation in response to increases in flow (flow-dependent dilation) could be reproducibly elicited in 18 of 23 dogs. The effect of intimal denudation (see later) on flow-dependent dilation was studied in 8 of these 18 dogs (Group 1). Studies in Group 2 (\( n = 6 \)) followed an identical experimental protocol except that the endothelium was damaged by hydrogen peroxide treatment (see later) instead of intimal denudation. The effect of alterations in vascular resting diameter on the magnitude of the subsequent flow-dependent dilat-
Preparation of the canine femoral artery in situ

Intra-arterial (i.a.) infusions of drugs could be performed alternately through the side branch distal or proximal to the diameter sensors. Pressure recordings (P) were obtained simultaneously using the remaining side branch catheter. To induce an increase in flow (as demonstrated by the chart records on the right), acetylcholine (ACh) was infused through the distal catheter, whereas pressure was recorded through the proximal catheter. Arrows indicate start of ACh infusion. By means of two snares (S) or pneumatic occluders, the vessel could be functionally isolated and perfused through the side branches. A flow-limiting stenosis could be induced by narrowing the distal snare.

Figure 1: Preparation of the canine femoral artery in situ. Flow (I) was recorded distally to the diameter sensors (D). Intra-arterial (i.a.) infusions of drugs could be performed alternately through the side branch distal or proximal to the diameter sensors. Pressure recordings (P) were obtained simultaneously using the remaining side branch catheter. To induce an increase in flow (as demonstrated by the chart records on the right), acetylcholine (Ach) was infused through the distal catheter, whereas pressure was recorded through the proximal catheter. Arrows indicate start of Ach infusion. By means of two snares (S) or pneumatic occluders, the vessel could be functionally isolated and perfused through the side branches. A flow-limiting stenosis could be induced by narrowing the distal snare.
dilator response had to be considered. Therefore, expiration was restricted during constant artificial ventilation (volume-controlled), which resulted in increased intrathoracic pressure and reduced venous return (Valsalva's maneuver). In this manner, we induced decreases in arterial pressure that were similar in magnitude and duration to those obtained during increases in flow.

To test and compare the reactivity before and after the endothelial-damaging procedures, the local vasomotor responses to 3-norepinephrine (levarterenol; Sigma), 1-10 μM, and nitroglycerin (Nitrolingual), 100-150 μM, were studied in random order. The vasoactive agents were applied topically from the adventitial side of the arterial wall in the area with the ultrasound crystals by continuous superfusion (superfusion rate, 1.5 ml/min) until a steady state was reached.

In three experiments with Group 1 and in two with Group 2, the vasomotor agonists (norepinephrine, 3 μg/kg/min; nitroglycerin, 1 5 μg/kg/min) were infused intra-arterially instead of being applied by adventitial superfusion. Although comparisons of vasomotor responses obtained by this technique before and after endothelial damage showed results qualitatively similar to those obtained with adventitial superfusion in the other animals, these data were not included in the evaluation of the vasomotor responses (see Results), since constant systemic hemodynamic conditions could not be maintained during intra-arterial infusions.

Intimal denudation or hydrogen peroxide perfusion were performed after the control determinations. The same determinations were repeated in random order after a 30-minute equilibration period.

The importance of resting vascular tone on the magnitude of flow-dependent dilation was investigated in Group 3, since this factor may be especially important during anesthesia. Different steady states of resting vascular diameter were induced by superfusing the vessel with Tyrode's solution containing norepinephrine (1-100 μM) or nitroglycerin (10-200 μM). At each of these resting diameters a standardized increase in flow (4.0 ± 0.3-fold) was induced by distal infusion of acetylcholine (1-2 5 μg/kg/min). At the end of the experiment, a maximal diameter was obtained by continuous superfusion with papaverine (100 μM) for 15 minutes. The maximal diameter was used to normalize the actual resting diameters.

**Statistical Analysis**

The amplitudes of outer diameter changes due to increases in flow were obtained by finding the difference between the baseline values and the maximum responses. No attempt was made to compensate for the concomitant decrease in pressure during peripheral vasodilation by acetylcholine. The absence of a flow-dependent dilation (passive, pressure-dependent changes of vascular diameter only) was verified by inducing flow increases at least four different times starting from different levels of resting vascular diameter. Hemodynamic parameters and vascular diameters between the two groups subjected to different techniques of endothelial damage (intimal denudation vs hydrogen peroxide treatment) were compared using Student's t test for unpaired data. Comparisons of resting vascular diameters, hemodynamic parameters, and vasomotor responses before and after endothelial damage within each group were performed using Student's t test for paired data.

To compare reactions (flow-dependent dilation and agonist responses) before and after endothelial damage, determinations were selected on the basis of congruent basal hemodynamic conditions and resting diameter. These determinations were presented in Table 3 and Figure 5, respectively. The selection was performed to exclude possible interdependencies of the magnitude of reactions and the resting vascular tone (see also Influence of Resting Diameter), which sometimes varied with time throughout the experiments. However, a flow-dependent dilation could not be elicited in all determinations after endothelial damage (n = 48), and the reactions to agonists (n = 27) were qualitatively similar to those in the selected determinations.

The data are expressed as means ± standard deviation (SD) unless otherwise stated. Dosages refer to the pure bases of the drugs.

**Results**

**Basal Hemodynamic Data**

Table 1 shows the basal hemodynamic data (control values after surgical preparation) for the three groups. Since there were no statistically significant differences between groups, the results obtained from control determinations in Groups 1 and 2 are presented together in the following section.

**Table 1** Basal Hemodynamic Data After Surgical Procedures in the Three Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MFAP (mm Hg)</th>
<th>MFAF (ml/min)</th>
<th>MOD (mm)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>141 ± 10</td>
<td>105 ± 37</td>
<td>4 74 ± 0 68</td>
<td>131 ± 19</td>
</tr>
<tr>
<td>2</td>
<td>140 ± 15</td>
<td>91 ± 17</td>
<td>4 32 ± 0 81</td>
<td>136 ± 20</td>
</tr>
<tr>
<td>3</td>
<td>138 ± 11</td>
<td>96 ± 20</td>
<td>4 60 ± 0 67</td>
<td>137 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SD. Statistical analysis revealed no significant differences between the groups. MFAP = mean femoral arterial pressure, MFAF = mean femoral arterial flow, MOD = mean outer femoral arterial diameter, HR = heart rate.
Dilator Response

Flow-dependent dilations were observed in 18 of 23 dogs. In three of five nonresponding dogs (with signs of trauma and/or prolonged surgical procedure), the acetylcholine response also could not be elicited. In the remaining two nonresponders, a weak, flow-dependent dilation (<30 μm) could be elicited initially and spontaneously ceased with time. In five of 18 dogs, a flow-dependent dilation could be observed in only one of the two arteries. A flow-dependent dilation was elicited at least twice in all responders (n = 28). During increases of flow (4.6 ± 1.9-fold), induced either by distal acetylcholine administration or arteriovenous shunt, local pressure decreased significantly (19 ± 7 mm Hg; p < 0.01) The vascular diameter also decreased passively (−34 ± 17 μm; p < 0.01) but started to rise above control values after a latency period of 42 ± 23 seconds. The maximum dilation (116 ± 91 μm; p < 0.01) was reached after 145 ± 23 seconds. A typical example of the flow-dependent dilation is shown in Figure 2.

In all responders, a decrease in blood pressure (31 ± 11 mm Hg; p < 0.01) alone, induced by Valsalva’s maneuvers, was not associated with vasodilation but rather resulted in significant passive decreases in diameters (−44 ± 21 μm; p < 0.01). Furthermore, the infusion of acetylcholine through the distal catheter alone did not elicit a vasodilation in the area of diameter sensors when the increase in flow was prevented by a flow-limiting stenosis, which excludes local effects of distally infused acetylcholine at the site of diameter crystals.

Influence of Resting Diameter

In Group 3, the maximal dilator response to comparable increases in flow varied considerably as a function of the actual resting diameter (Figure 3). The dilator responses elicited by the increases in flow were abolished at extremely dilated as well as contracted states. They reappeared at resting diameters between these limits. The mean maximal diameter obtained by papaverine at the end of the experiments amounted to 5.04 ± 0.40 μm.

Local Responses to Acetylcholine Effects and Adventitial Superfusion of Vasomotor Agonists

In Groups 1 and 2, a vasodilation (152 ± 114 and 143 ± 54 μm respectively; p < 0.01 vs control in both groups) was observed in response to proximally infused acetylcholine while the increase in flow was prevented by distal stenosis, demonstrating a functionally intact endothelium (Figures 4 and 5). Infusion of Tyrode’s solution or distal occlusion alone did not affect the vascular diameters. Topical (superfusion) application of norepinephrine elicited a significant vasocostriction (298 ± 163 and 260 ± 134 μm; p < 0.01; see Figure 5), while opposing effects were obtained by superfusion with nitroglycerin, which elicited a significant vasodilation (158 ± 143 and 156 ± 112 μm; p < 0.01; see Figure 5). Topical application of the drug did not induce systemic effects as obtained from simultaneous diameter and pressure recordings in the contralateral artery. Temperature recorded with thermocouples (n = 4) at the adventitial side during superfusion experiments revealed a maximum difference of 1.9°C compared with intraluminal blood temperature, which was always above 37°C.
The protocol was discontinued in two of the eight dogs of Group 1, because of the virtual absence of vascular response to the vasoactive agonists. This functional observation correlated with microscopic signs of extensive trauma within the media, which excluded investigation of selective endothelial damage. Although in some animals there was a tendency for the mean resting vascular diameter to increase after intimal denudation, the alterations of vascular diameter and the ratio of diameter to pressure amplitudes were not statistically significant (Table 2). The diameter responses to both nitroglycerin and norepinephrine at comparable initial diameters after the equilibration period also were not significantly altered (see Figures 4 and 5), which indicates that vasoconstrictor and vasodilator capacities were preserved. In contrast, the flow-dependent dilation could no longer be elicited (Table 3). The vasodilator response to proximally infused intra-arteri-
al acetylcholine (even at 10-fold higher concentrations than those used during control conditions) also was completely abolished after intimal denudation (see Figures 4 and 5).

Effects of Hydrogen Peroxide Treatment

The hydrogen peroxide treatment did not affect significantly the morphological intactness of the vascular intima (examined by light microscopy). Neither resting vascular diameters nor the pulse pressure–induced diameter amplitudes were significantly altered, either immediately after hydrogen peroxide treatment or after the 30-minute equilibration period (see Table 2). The norepinephrine-induced and nitroglycerin-induced changes of vascular diameter were not significantly different from control values (see Figure 5). In contrast, the vasodilator response to proximally applied acetylcholine was attenuated significantly by 27.4 ± 19.2% (p < 0.01; see Figure 5), and no vasodilator responses to flow increases could be observed after hydrogen peroxide treatment (see Table 3). Sham treatment (perfusion of the contralateral arterial segment with Tyrode’s solution) did not prevent the vasodilator responses to increases in flow (n = 3).

Discussion

The major finding in this investigation is that the vasodilator response to increases in flow was critically dependent on the presence of a functionally intact endothelium, as evidenced by the complete abolition of the flow-dependent dilation by each of two techniques used to offset the endothelial cell function. Endothelial cells in culture are known to react to alterations in hydromechanic forces (as would occur during increases in blood flow) by altering metabolism and intracellular morphological structures (stress fibers). The present results indicate that, under in vivo conditions, the endothelium reacts to increases in flow by generating a vasodilator signal.

A peripheral conducting mechanism (ascending dilation), proposed by early workers in this field, was convincingly excluded by experiments demonstrating the persistence of flow-dependent dilation after distal transsection of the artery. Our experiments using an arteriovenous shunt are in agreement with these findings. A substantial contribution of myogenic relaxation (Bayliss effect) to flow-dependent dilation can also be excluded. In the control experiments (employing Valsalva’s maneuver) pressure drops of a magnitude similar to those obtained during the increases in flow did not elicit an active vasodilation. This finding is in accordance with the results of Lie et al. and Gerova et al., who used different approaches to alter (or maintain) transmural pressure.

Removal of endothelial cells was verified by postmortem microscopic examination, as well as functionally by the absence of local vasodilator response to proximally applied acetylcholine. This acetylcholine response has been demonstrated to be an endothelium-mediated reaction in various types of isolated vessels in saline media as well as in the blood-perfused canine femoral artery in situ. In the absence of endothelial cells, acetylcholine is reported to elicit no vasmotor response or small contractions at concentrations higher than those used in this study. Although acetylcholine (infused distally) also was used to induce increases in flow, we excluded an effect on flow-dependent dilation by recirculation or retrograde leakage of the distally infused drug.

It is important to note that the intimal denudation abolished the local acetylcholine response and the flow-dependent dilation without detectably altering the vasmotor effects of nitroglycerin and norepinephrine. This finding indicates that the vessel reactivity was grossly unchanged after intimal denudation. In addition, neither the resting diameter nor the vascular wall mechanics, as obtained from the diameter amplitude/pulse pressure ratio, were significantly affected by the removal procedure. This finding argues against generalized damage to the vessel wall and, therefore, excludes trauma of the media as a major cause for the disappearance of the flow-dependent dilation.

Nevertheless, intimal denudation in vivo necessarily
interferes with the input of vasoactive signals to vascular smooth muscle in a dual manner. First, the lack of endothelium-derived vasodilator signals may unmask the direct smooth muscle constrictor effects of substances acting simultaneously on endothelium and smooth muscle (e.g., serotonin). Second, some substances released from platelets and white blood cells adhering to the traumatized surface (e.g., adenosine 5'-monophosphate (ADP) and free radicals) may reduce smooth muscle tone in this area, which would explain the lack of a significant change in resting vascular diameter after intimal denudation in vivo that has also been reported by Angus et al. Although the sum of these effects could interfere with smooth muscle responsiveness, it is difficult to conceive that such influences would selectively affect the dilation induced by acetylcholine and by flow increases but not the smooth muscle response to the other vasomotor stimuli applied.

An experimental approach that suppresses endothelial cell functions without concomitant destruction of endothelial cells would prevent some of the aforementioned problems with intimal denudation. Among the known agents that suppress endothelial cell function, hydrogen peroxide is an attractive candidate for two reasons. It is endogenously produced by a variety of cells, and it appears to be a major cytotoxic agent in various pathophysiological conditions. Furthermore, it has been demonstrated in endothelial cells in culture that hydrogen peroxide (either exogenously applied or released from stimulated white blood cells) can selectively, and dose-dependently, alter cellular functions without signs of cellular decomposition. Recently, a brief (4 minutes) treatment of endothelial cells in culture with hydrogen peroxide was shown to be sufficient to suppress secretory endothelial cell responses to various stimuli such as ADP, bradykinin, and the calcium ionophore A23187 even 20 minutes later.

In the present experiments, hydrogen peroxide treatment (with a time schedule similar to the described in vitro experiments) did not induce significant morphological alterations of the intimal cell layer. Despite the reported smooth muscle relaxing property of hydrogen peroxide, no persistent effects on vascular smooth muscle were detectable after the equilibration period with respect to the reactions to vasomotor agonists. However, the acetylcholine response was significantly diminished compared with control values, which indicates some degree of functional damage to the endothelium. The flow-dependent dilation was completely abolished after hydrogen peroxide treatment.

The significance of the endothelial cell response to acetylcholine stimulation with respect to other endothelial cellular functions has not yet been established. However, it is possible that the reaction of endothelial cells to an increase of flow is much more sensitive to damaging effects than the acetylcholine response. This view is supported by the finding that in two dogs there was a response to acetylcholine even after spontaneous disappearance of the flow-dependent dilation, whereas the opposite (i.e., flow-dependent dilation without demonstrable response to acetylcholine) was never observed.

The nature of the endothelium-derived signal released by "flow-stimulated" endothelial cells is still unknown. Release of a humoral endothelium-derived relaxant factor (EDRF) has been demonstrated after acetylcholine stimulation. Such a humoral EDRF also may be responsible for smooth muscle relaxation in response to flow. The delayed onset of the flow-dependent dilation as well as its slow decline after cessation of the stimulus suggests that either the transformation of the flow-related stimulus (viscous drag?) or the production and extrusion of a humoral factor involves slowly developing steps possibly associated with physicochemical alterations of the cell membrane or with changes in enzyme activities.

The observed diameter dependency suggests that the putative factor released by the endothelial cells does not elicit an all or none response in the vascular smooth muscle during flow increases. It appears to modulate the vascular tone with the exception of states of extreme vasoconstriction. From the present experiments it cannot be distinguished whether the EDRF is inactivated under these conditions by high local concentrations of norepinephrine or whether the vasodilator potency of the EDRF (or its concentration) is too low to overcome smooth muscle contraction. The maximum levels of diameter reduction induced in our study were comparable to those obtained by profound sympathetic stimulation in situ.

It is conceivable that circulating EDRF released at the luminal side of intact endothelium, proximal to the denuded area, can exert a dilator response in the denuded segment. However, there is evidence that a plasma factor (or factors) may inactivate the EDRF. In earlier experiments on flow-dependent dilations in coronary arteries of conscious dogs, it was demonstrated that the flow-dependent dilation is not due to endothelial release of prostaglandins, since inhibitors of cyclooxygenase did not suppress the flow-dependent dilation. Similarly, α-blockade, β-blockade, and pretreatment with antihistaminic agents did not interfere with the flow-dependent dilation, which appears to exclude mechanisms acting through the respective receptors.

In conclusion, our results suggest that endothelial cells play a decisive role in the vasodilator response to increases in flow. Therefore, the endothelium can modulate vascular tone of conduit vessels to adapt vessel calibers to sudden and chronic changes in tissue perfusion requirements. These findings further suggest that a tonic, flow-related modulation of the vascular smooth muscle activity by the endothelium does exist, the alterations of which may be important in numerous cardiovascular diseases.

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