Alterations in Flow-Dependent Vasomotor Tone in Spontaneously Hypertensive Rats

Hong Ying Qiu, Daniel Henrion, Bernard I. Levy

Abstract We studied the effect of endothelium on the flow-induced response of conductance arteries and the resistance arterial network in an in situ model of perfused mesenteric artery in normotensive Wistar-Kyoto and spontaneously hypertensive rats. The mesenteric network was perfused with a Tyrode’s albumin solution. The diameter of a conductance mesenteric artery was measured using a video camera system, and mesenteric pressure was recorded in a collateral artery. The preparation was perfused at 0.2, 2, and 4 mL/min, and flow-diameter-pressure relations were established (1) under control conditions, (2) during local inhibition of nitric oxide by topical application of N\textsuperscript{N}-nitro-L-arginine methyl ester (L-NAME) (1 mmol/L), and (3) after endothelium removal (CO\textsubscript{2} drying). In normotensive rats, L-NAME decreased conductance artery diameter by 12±2% (P<.01) at 0.2 mLMin and 3.3±1.9% (P<.05) at 2 mL/min. In hypertensive rats, L-NAME did not modify mesenteric diameter. Endothelium removal markedly increased arterial resistance in both strains and decreased conductance artery diameter in normotensive rats (10.3±3%, P<.05 at 0.2 mL/min and 4.2±2%, P<.05 at 2 mL/min) but not in hypertensive rats. The present study suggests that the endothelium plays a similar role in the control of mesenteric resistance in both strains and that there is a significant diameter-flow dependency affected by both endothelium removal and inhibition of nitric oxide synthesis in conductance mesenteric arteries from normotensive but not from hypertensive rats. (Hypertension. 1994;24:474-479.)

Key Words • vasodilation • mesenteric arteries • nitric oxide • hypertension, spontaneous • endothelium synthesis on flow-induced dilation in in situ mesenteric resistance and conductance arteries of spontaneously hypertensive rats (SHR) and normotensive control Wistar-Kyoto (WKY) rats.

Methods

Experimental Model

All experiments were performed on 22 twelve-week-old WKY rats and 22 age-matched SHR. All rats were obtained from Centre D’Elevage R. Janvier (St-Berthevin, France). The investigation conformed with the guidelines for the care and use of laboratory animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1985). Rats were anesthetized with sodium pentobarbital (50 mg/kg IP), and a medial laparotomy was performed. The last loop of the small intestine was exposed and placed in a container that allowed superfusion of the preparation (Fig 1). The preparation was immediately irrigated with buffered Tyrode’s solution gassed with 95% O\textsubscript{2}/5% CO\textsubscript{2} at 37° to 38°C. A 3-mm-long segment of a second-generation mesenteric artery (external diameter, approximately 500 \textmu m) was gently dissected free of fat and connective tissue under a binocular microscope (Microcontrol) (Fig 2). The mesenteric artery and vein were separated to obtain a clear image of the isolated arterial segment. A video camera (Microcontrol) mounted on the binocular lens allowed recording and analysis of the images of the isolated arterial segment. The total magnification of the optical system was ×160.

A polyethylene catheter (external diameter, 0.6 mm; internal diameter, 0.28 mm) was placed in the first-generation branch of the mesenteric artery and connected to a syringe infusion pump (Harvard Apparatus) driving a 20-mL Becton Dickinson syringe. The mesenteric arterial bed was perfused using Tyrode’s albumin (4%) solution at 0.2, 2, and 4 mL/min. Another 10-cm-long catheter, connected to a pressure transducer (Gould P10EZ, Spectramed) and recorder (Gould), was placed in an arterial branch located downstream of the observed segment of artery for recording of mesenteric arterial pressure. Each flow rate was maintained for 2 minutes to obtain steady-state arterial diameters and intraluminal pres-
The observed segment of mesenteric artery of 10 WKY rats and 10 SHR was given a topical application of Tyrode's albumin solution (0.2 mL/min) for 20 minutes. During this time the mesenteric bed was perfused with the Tyrode's albumin solution (0.2 mL/min). Diameters and pressures were recorded at flow rates of 0.2, 2, and 4 mL/min. For each rat we verified that the topical application of L-NAME did not produce any change in the intraluminal mesenteric pressure continuously recorded.

**Endothelium Removal**

The mesenteric network of the second group (10 WKY rats and 10 SHR) was injected with 2 mL CO₂ for 30 seconds via a syringe for gas drying. The studied artery was then perfused (0.2 mL/min) with the Tyrode's albumin solution, and the integrity of the smooth muscle layer was verified by constriction with phenylephrine (10 μmol/L) unaffected by gas drying; furthermore, the absence of endothelium was confirmed by the lack of a vasodilator effect of topically applied acetylcholine (10 μmol/L) on the vessel preconstricted by phenylephrine. Twenty minutes later, diameters and intraluminal pressures were recorded at flow rates of 0.2, 2, and 4 mL/min under steady-state conditions.

Preliminary experiments showed that the order of flow rate perfusion, increasing from 0.2 to 4 mL/min or decreasing from 4 to 0.2 mL/min, had similar effects on the recorded arterial pressures and diameters.

**Drugs**

L-NAME, phenylephrine, and acetylcholine were purchased from Sigma Chemical Co.

**Statistical Analysis**

Results are expressed as mean±SEM. The experimental design allowed the use of two-way ANOVA with repeated measurement (flow rates) to show differences caused by strain or experimental condition. Comparisons of pressure-diameter relations between different experimental conditions were made using ANCOVA²¹ for repeated measures with BMDF software (University of California at Los Angeles).

**Results**

Fig 3 shows the mesenteric flow-pressure, flow-diameter, and pressure-diameter relations in situ perfused networks of WKY rats and SHR under control conditions. Intraluminal mesenteric pressure increased linearly with flow in both WKY rats and SHR (Fig 3A). Although the absolute pressures for a given flow rate were significantly higher in SHR than WKY rats (P<.01), the relative pressure increases with flow rate were similar in both strains. There were no significant differences in the flow-diameter relations (Fig 3B) or in the pressure-diameter relations (Fig 3C) between WKY rats and SHR.

**Effects of Topical L-NAME**

Topical application of L-NAME (1 mmol/L) did not alter mesenteric arterial pressure compared with control conditions for both strains (data not shown). In WKY rats, the topical application of L-NAME induced significant decreases in mesenteric diameter by 12±2% (P<.01) at 0.2 mL/min and 3.3±1.9% (P<.05) at 2 mL/min (Fig 4). In SHR, L-NAME did not modify the diameter-flow relation. When compared for the whole range of flow rates, there was a significant difference (P<.01) in the diameter decreases induced by L-NAME in the WKY and SHR groups. These results suggest that topical application of L-NAME affected the flow-induced vasodilation in WKY but not in SHR mesenteric conduit arteries.
Effect of Endothelium Removal

Endothelium removal significantly increased the resistance of the perfused mesenteric arterial bed in both rat strains ($P<.01$ in WKY rats, $P<.001$ in SHR). There was no significant difference in the increase in resistance between WKY rats and SHR (Fig 5). In WKY rats, the increase in mesenteric resistance was accompanied by significant decreases in mesenteric conduit artery diameter by $10.3\pm2.2\%$ ($P<.05$) at $0.2\text{ mL/min}$ and by $4.2\pm2.3\%$ ($P<.05$) at $2\text{ mL/min}$ (Fig 6). There was no significant difference between the mesenteric diameter reduction induced by topical application of L-NAME and by endothelium removal in WKY rats. In SHR, although the increases in resistance caused by endothelium removal were similar to those in WKY rats, the diameter-flow relation of mesenteric conductance arteries was not affected by endothelium removal (Fig 6).

The Table summarizes the results obtained under different experimental conditions in WKY rats and SHR.

Discussion

The main findings of the present study are (1) mesenteric peripheral fluid resistance was greater in SHR than WKY rats under control conditions, so mesenteric arterial pressure was higher in SHR at identical perfusion flow rates; (2) the diameter of large (approximately $500\,\mu\text{m}$) conductance mesenteric arteries in WKY rats and SHR was not significantly different when perfused at the same flow rates; (3) endothelium removal induced marked and similar increases in mesenteric fluid resistance between WKY rats and SHR (Fig 5). In WKY rats, the increase in mesenteric resistance was accompanied by significant decreases in mesenteric conduit artery diameter by $10.3\pm2.2\%$ ($P<.05$) at $0.2\text{ mL/min}$ and by $4.2\pm2.3\%$ ($P<.05$) at $2\text{ mL/min}$ (Fig 6). There was no significant difference between the mesenteric diameter reduction induced by topical application of L-NAME and by endothelium removal in WKY rats. In SHR, although the increases in resistance caused by endothelium removal were similar to those in WKY rats, the diameter-flow relation of mesenteric conductance arteries was not affected by endothelium removal (Fig 6).

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resistance in both strains; and (4) in normotensive WKY rats, the basal vascular diameter and flow-diameter dependency of the conductance mesenteric artery were significantly and similarly reduced by topical L-NAME and endothelium removal. In contrast, in SHR the diameter-flow relation of the conductance mesenteric artery was not modified by topical L-NAME or endothelium removal.

The in situ model of the perfused mesenteric arterial bed presents several methodological advantages: (1) The normal length and geometry of the arteries are retained throughout the experiment; (2) endothelium integrity is not altered; and (3) solutions can be perfused and/or superfused, and therefore the behavior of a conductance mesenteric artery and the resistance of the mesenteric network from the same animal can be studied simultaneously under the same experimental conditions.

However, the resistance of the mesenteric circulation is calculated from flow and pressure measurements, therefore allowing an indirect estimation of mesenteric vascular resistance. Several groups have reported that peripheral resistance is located not only in the distal microarteries but that a significant portion of the resistance is due to macroarteries.22-25 Furthermore, it has been suggested that the macroarteries contribute to the increased peripheral resistance observed in anesthetized hypertensive rats.22,24 Christensen and Mulvany26 recently reported that in normotensive and anesthetized SHR, approximately one third of the resistance of the mesenteric bed lies in macroarteries. In addition, the perfusion pressure recorded in the larger mesenteric arteries reflects the resistance of the overall mesenteric bed (macroarteries and microarteries) calculated as the pressure-flow ratio.

It would be of great interest to measure simultaneously the diameter in large conduit and small resistance arteries. However, the mesenteric resistance arteries are located on the wall of the intestine, and we could not simultaneously control flow and measure diameter in such resistance arteries and in large conduit arteries. Therefore, we cannot obtain similar, physiological, and controlled experimental conditions in large and small arteries. We can remark that the few available in vivo references to the mesenteric bed give data in arteries larger than 350 μm in diameter at low transmural pressure.27

The functional significance of flow-induced dilation is determined by its magnitude, which varies with changes in vascular tone.5,28,29 Because the conduit arteries contribute little to total hydrodynamic resistance of resting muscle, the increase in conduit arterial diameter induced by a large increase in flow is generally small.29 However, the diameter of coronary arterioles has been reported to increase by as much as 30%, and the diameter of upstream larger coronary arteries increases by only 4%.28 In agreement with previous studies, the diameter of mesenteric conductance arteries perfused at various flow rates increased under control conditions by 5% in WKY rats and 4% in SHR (present study). These increases in diameter were probably not large enough to cause an effective decrease in the pressure drop along the artery during increases in flow.

The arterial mesenteric pressure increased immediately after each increase in flow rate and became stable when a steady-state diameter was reached, indicating a positive interaction between flow-induced dilation and increases in transmural arterial pressure. It has been reported that increased shear stress caused by increases in flow rate or viscosity causes the release of endothelial vasodilator substances, eliciting arterial dilation.38 The transmural pressure-induced myogenic response, which is mostly endothelium independent,27,30 also interferes with changes in diameter. A rapid increase in flow rate causes an increase in transmural pressure that induces in small resistance arteries a myogenic response, which is a “positive-feedback pressure-sensitive” mechanism.29,31 Dilatation mediated by the endothelium in response to increased intraluminal flow provides an opposing feedback mechanism.27 It has been reported that in the presence of flow, the pressure-diameter relation of intact resistance arteries was modified and the magnitude of myogenic responsiveness attenuated.32 Similarly, Griffith,31 working on a rat intact mesenteric preparation, reported that myogenic responses occurred only in the presence of hemoglobin, an inhibitor of NO activity. In the present study, endothelium removal increased mesenteric resistance markedly and similarly in WKY rats and SHR. This is the first demonstration that the endothelium had a major dilating function under flow conditions in hypertensive rats.

Vascular resistance did not change in response to topical L-NAME in both strains, probably because L-NAME did not reach resistance vessels in the non-dissected mesenteric network. Therefore, the effect of L-NAME on the exposed mesenteric artery segment could be studied under basal and L-NAME conditions at similar levels of intraluminal pressure.

In WKY rats, the flow-diameter relation was significantly shifted downward by L-NAME, suggesting an inhibition of flow-induced dilation in large conductance mesenteric arteries. Inhibition of NO release has a

<table>
<thead>
<tr>
<th>Strain</th>
<th>Diameter, μm</th>
<th>Pressure, mm Hg</th>
<th>Changes In Diameter, %</th>
<th>Changes In Pressure, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td>518±11</td>
<td>91±5</td>
<td>-3.3±1.9</td>
<td>+0.55±0.7</td>
</tr>
<tr>
<td>SHR</td>
<td>536±14</td>
<td>106±3.4</td>
<td>+1.7±1.2</td>
<td>-4.2±2*</td>
</tr>
</tbody>
</table>

WKY indicates Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; and L-NAME, N'nitro-L-arginine methyl ester. Perfusion flow was 2 mL/min.

*P<.05, †P<.01, compared with control conditions.

‡P<.05, SHR compared with WKY rats under the same experimental conditions.
similar effect in vitro in rabbit ear arteries and porcine coronary arteries. Shear stress–dependent dilation can be mediated by multiple endothelium-dependent responses involving the release of NO, endothelium-derived hyperpolarization factor, and/or prostacyclin. However, in the present study we decrease in flow-induced dilation after endothelium removal was similar to that observed under topical application of L-NAME. This suggests that the flow-induced NO release mediates most of the diameter–flow dependency in large mesenteric arteries of WKY rats.

Arterial diameter was significantly reduced by removal of the endothelium, despite the consecutive increase in perfusion pressure larger than that recorded with intact endothelium. This decrease in diameter may reasonably be attributed to the loss of endothelium-dependent responses to flow.

In SHR under control conditions, the flow–diameter relation in mesenteric conduit arteries was similar to that in WKY rats. The perfusion pressure in SHR was higher than in WKY rats at a given flow rate, suggesting that the presence of the endothelium was essential for flow–diameter regulation in SHR. However, essential hypertension has been characterized by increased vascular resistance, generally attributed to morphological and functional changes in vascular smooth muscle contractility and in endothelium functions. The endothelium-dependent responses to acetylcholine of vessels from hypertensive animals are attenuated, whereas the endothelium-independent responses remain intact or even increased.

The lower response to acetylcholine is not likely to be due to a reduced release of endothelium–derived relaxing factor but to the simultaneous release of a contracting prostanoid from the endothelial cells. However, hemodynamic studies indicate that the basal activity of NO is similar in SHR and WKY rats, although NO appears to contribute differentially to tone in the different vascular beds. In a previous study, we found that the cyclic GMP content of aortic walls from SHR was greater than from WKY rats. In the same way, Fukuda et al. demonstrated that the production of cyclic GMP in response to sodium nitroprusside infusion in the mesenteric bed was higher in SHR than in WKY rats. We recently observed that flow-induced release of cyclic GMP in the mesenteric bed was significantly larger in SHR than in WKY rats (Reference 46 and unpublished data). These observations suggest that the production of NO released by the endothelium in large arteries is probably overactivated in SHR. The present results showed that topical L-NAME did not change the flow–diameter relation in conductance mesenteric arteries of SHR. This suggests either that L-NAME does not affect flow-induced NO release or that the flow dependency of conductance artery diameter is not predominantly controlled by NO release in SHR.

Endothelium removal results in similar increases in perfusion pressures in mesenteric arterial bed of both SHR and WKY rats. This suggests that the endothelium plays an important role in the control of the vasomotor tone of the mesenteric resistance arteries of WKY rats and SHR. However, neither L-NAME nor endothelium removal affected the flow–diameter relations in SHR mesenteric conduit arteries, suggesting that there is no endothelium-dependent regulation of diameter by flow in SHR. Hence, flow-induced dilation of SHR conduit mesenteric arteries not only is independent of NO release but is also endothelium independent. The mechanism responsible for this impairment is not clear, but it could be related to an increase in Ca2+-dependent K+ currents in arterial smooth muscle cell membranes of hypertensive rats. Another hypothesis is that a major part of flow–mediated dilation in SHR depends on the media. It has been reported that a portion of the flow-mediated dilation is dependent on the media of arteries. In conclusion, the present study suggests that in the in situ perfused intact mesenteric network from normotensive rats and SHR, (1) the endothelium plays a similar role in the control of mesenteric arterial bed resistance in both strains, and (2) there is a significant diameter–flow dependency affected by both endothelium removal and inhibition of NO synthase in large conductance mesenteric arteries from normotensive but not from hypertensive rats.

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References
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