Vasomotor responses in chronically hyperperfused and hypoperfused rat mesenteric arteries

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Pourageaud, Fabrice, and Jo G. R. De Mey. Vasomotor responses in chronically hyperperfused and hypoperfused rat mesenteric arteries. Am. J. Physiol. 274 (Heart Circ. Physiol. 43): H1301–H1307, 1998.—We evaluated the reactivity of small arteries after remodeling induced by elevated or reduced blood flow. In 6-wk-old rats, every other first-order side branch of the superior mesenteric artery was ligated near the bifurcation of second-order branches. Four weeks after surgery, vessels that had been exposed to high flow (HF) or low flow (LF) were isolated and mounted in a pressure myograph at 100 mmHg and were compared with vessels from sham-operated rats (Sham). In HF: 1) basal lumen diameter was increased; 2) sensitivity to norepinephrine, arginine vasopressin, and perivascular nerve stimulation was not modified; 3) maximal constrictor responses (A diameter) to these stimuli and 125 mM K+ were increased; and 4) sensitivity and maximal dilator responses to sodium nitroprusside, acetylcholine, and flow were not modified. In LF: 1) basal diameter was reduced; 2) sensitivity to constrictor stimuli was not altered; 3) maximal responses to all vasoconstrictors except arginine vasopressin were reduced; and 4) sensitivity but not maximal dilator responses to sodium nitroprusside and acetylcholine was reduced. During acute flow-induced dilatations, lower shear stress was maintained in HF (48 ± 7 dyn/cm²) than in Sham (63 ± 10 dyn/cm²), but no shear stress regulation was observed in LF. These observations indicate that arterial structural responses to altered blood flow are accompanied by modified reactivity of the arterial smooth muscle, which entails changes in responsiveness to neurogenic and endothelium-dependent stimuli.

Arteries are sensitive to acute and chronic changes in blood flow. The frictional force exerted by flow influences endothelial release of various vasoactive agents (4, 6, 14, 25). Thereby arterial lumen diameter is acutely adjusted in order to keep wall shear stress constant (14, 23). When changes in blood flow are chronically maintained, the structure of the vessel is modified (12, 16–18, 30). For instance, in rat mesenteric small arteries, chronic hyperperfusion leads to outward hypertrophic remodeling, and chronic hypoperfusion results in inward hypotrophic remodeling (22, 31, 32). The relationship between arterial vasomotor and structural responses to changes in blood flow largely remains to be established.

Several endothelium-derived vasoactive agents can modify arterial structure on the long run through effects on the growth and turnover of endothelial and vascular smooth muscle cells (3, 4, 26). Vascular smooth muscle cells may become more or less sensitive to mediators produced at a reduced or increased level, respectively, during the remodeling process.

MATERIALS AND METHODS

Experimental groups. Six-week-old male Wistar-Kyoto rats (Central Animal Facilities, Universiteit Maastricht), which weighed 141 ± 10 g, were randomly divided into two groups of seven animals each. In the first group, surgery was performed to persistently modify blood flow in mesenteric arteries (22). In the second group, a similar surgical intervention was performed but without flow modification (Sham). The experimental and sham groups were used 4 wk later. All rats were kept in individual cages on a 12:12-h light-dark cycle and had free access to standard rat food and tap water. The experimental protocols were approved by the Ethical Committee for Animal Welfare of the Universiteit Maastricht.

Surgical intervention. Persistent changes in mesenteric artery blood flow were induced as previously described (22). In brief, in 6-wk-old rats that had been anesthetized with pentobarbital sodium (60 mg/kg ip) loops of the small intestine were exteriorized through a midline abdominal incision. Every other first-order side branch of the superior mesenteric artery was ligated distally near the bifurcation of second-order branches using 6–0 surgical thread. After 12 vessels had been ligated and the intermittent parallel vessel had been left patent, the gut was returned to the abdominal cavity. The abdominal muscle layers and skin were sutured with surgical sutures.

This surgical model is based on the following anatomic characteristics of the rat mesenteric arterial bed. The superior mesenteric artery gives rise to ~25 first-order side branches. Each of these has a number of small branches along its length that perfuse the perivascular fat and mesentery,
but most of the flow is directed toward the small intestine. Near the gut, the arterial trees perfused by the mesenteric artery branches are interconnected. As a result of these characteristics, distal ligation of a first-order mesenteric artery reduces the flow through the vessel to a low but still significant level and results in an elevation of the flow through parallel nonligated arteries.

We previously reported (22) that flow in first-order mesenteric artery branches averages 1) 0.30 ± 0.03 ml/min in 6-wk-old rats; 2) 0.26 ± 0.02 ml/min in 10-wk-old rats that had been subjected to sham surgery; 3) 0.03 ± 0.01 and 0.05 ± 0.01 ml/min at 30 min and 4 wk after distal ligation, respectively; and 4) 0.51 ± 0.06 and 0.61 ± 0.08 ml/min at 30 min and 4 wk, respectively, in the intermittent parallel nonligated vessels. The intervention that clearly resulted in marked persistent alterations in local mesenteric arterial blood flow did not alter aortic blood pressure or the gain in body weight of the animals during the 4-wk experimental period.

Pressure myograph experiments. Four weeks after surgery, rats were killed by a sharp blow on the back of the head and exsanguination, and segments of first-order mesenteric arteries (4–5 mm long) were isolated. From each experimental animal, vessels that had been exposed to low flow (LF) and vessels that had been exposed to high flow (HF) were dissected. From Sham rats, corresponding control segments were taken. In all cases, the vessel segment was obtained halfway between the superior mesenteric artery and the bifurcation of the vessel. Thus LF were obtained proximally from the gut, and Sham were studied.

All preparations were mounted in an arteriographic system (Living Systems Instruments, Burlington, VT) in which wall thickness and internal diameter could be continuously monitored, while intraluminal pressure and flow were controlled (10). Segments were cannulated at both ends with a glass micropipette (internal diameter at tip 200 µm), tied to the pipettes with 11–0 surgical suture, and filled with filtered physiological salt solution (PSS) containing 1% albumin. Vessels were kept in a bath filled with 7 ml PSS that was kept at 37°C and oxygenated with 5% CO2-95% O2. In the absence of flow, the pressure was slowly raised to 150 mmHg. The distance between the pipettes was adjusted by positioning the movable cannula to prevent warping, axial compression, or stretch of the vessel. Thereafter, the pressure was set at 100 mmHg, and the preparation was allowed to equilibrate for 1 h in the absence of flow. The arteriographic system was placed on the stage of an inverted microscope (Nikon, Japan) equipped with a black-and-white videocamera (Stemmer, Germany). An electronic system (Living System Instrumentation) analyzed the signals obtained from the video image and continuously determined the internal diameter and wall thickness of the vessel. These parameters were recorded together with intraluminal pressure using a chart recorder.

Electrical field stimulation. Constant-current pulses (85 mA, 2 ms, 0.25–16 Hz) of alternating polarity were delivered by a stimulator (Technical Services, Universiteit Maastricht, The Netherlands) and passed between two platinum electrodes placed 4–5 mm along the vessel segment. Constrictor responses to electrical field stimulation could be blocked completely by tetrodotoxin (0.1 µM) and were reduced to <20% in the presence of prazosin (0.1 µM). They were therefore attributed to stimulation of perivascular sympathetic nerves.

Intraluminal flow. Resistances of proximal and distal pipettes were balanced before the experiments. A pressure transducer was placed upstream from the proximal pipette and downstream from the distal pipette. Intraluminal flow was measured with a flowmeter (model F-4001, Gilmont Instrument) connected to the distal pressure transducer. The proximal-to-distal pressure gradient was controlled by changing the downstream pressure in order to keep the intraluminal pressure at 100 mmHg (23). Intraluminal flow was increased from 0 to 1,000 µl/min in 100 µl/min steps. Each flow step was applied for 2–3 min.

Experimental protocols. After the equilibration period, constrictor responses were studied in the absence of flow by recording the decrease in the internal diameter in response to high potassium solution (125 mM) followed by extraluminal addition of norepinephrine (NE, 10 µM). After the preparations were washed with PSS until complete dilatation was accomplished, a frequency-response curve for electrical field stimulation was constructed (0.25–16 Hz) in the presence of cocaine (1 µM), an inhibitor of the neuronal reuptake of NE. After washing was completed, a concentration-response curve was constructed by cumulative addition of NE (0.01–10 µM) to the bath solution in the presence of cocaine (1 µM). When maximal constriction had stabilized, intraluminal flow of albumin-PSS was started (0–1,000 µl/min). When maximal flow-induced dilatations had been obtained, intraluminal flow was stopped and the maximal constrictor response to NE was usually restored within 3–4 min. Then acetylcholine (ACH, 0.01–10 µM) was administered extraluminally to evaluate the dilator effect of this endothelium-dependent agonist (23). After exposure to these agonists, the preparation was washed with PSS for at least 30 min before the next application of drugs. A cumulative concentration-response curve for arginine vasopressin (AVP, 0.01–100 nM) was constructed. We included this potent vasconstrictor agonist because its action is, unlike that of high K+, electrical field stimulation, and NE, not related to sympathetic nerves. During maximal constriction induced by the peptide, endothelium-independent dilatation was evaluated with the exogenous nitric oxide (NO) donor sodium nitroprusside (SNP, 0.01–100 µM).

Quantification of vasomotor responses. Effects of vasconstrictor stimuli were quantified as absolute and percent changes in lumen diameter and as changes in circumferential wall stress (WS) in order to normalize findings for differences in basal diameter and thickness between experimental groups. WS was defined as wall tension (T) divided by thickness (h) with T = P · D/2, in which P represents intraluminal pressure, and D is internal diameter. Effects of dilator stimuli were expressed as percentages of the preconstriction. To evaluate the sensitivity to agonists, the EC50, i.e., the concentration producing 50% of the maximal effect, was determined by interpolation on a least-square sigmoidal fit of individual concentration-response curves.

Wall shear stress (WSS) was calculated with the formula, WSS = 4η · Q / (πr2), where η is the viscosity of the albumin-PSS (0.0064 dyn · s · cm−2) (23), Q is flow (ml/s), and r is the vessel radius.

Histology and morphometry. At the end of each in vitro experiment, which lasted ~5 h, preparations were fixed at 100 mmHg in the pressure myograph system using 4% formaldehyde in phosphate-buffered saline. The vessels were maintained overnight in this fixative, stored in ethanol, and embedded in paraffin. Cross-sections (4 µm) were stained with Lawson's solution (Boom BV, Meppel, The Netherlands) to visualize the elastic laminae. Video images were generated from the cross-section using a Zeiss axioskope (Zeiss, Germany) and a standard CCD camera (Sony, Japan). Internal and external medial circumference, demarcated by the inter-
nal and external elastic lamina, were measured (Sigma Scan, Jandel Scientific, Corte Madera, CA). From these values, media cross-sectional area, internal radius, and media thickness were calculated for each section (1).

Biocompilation analysis. Separate LF and HF preparations and Sham vessels were used to estimate the density of sympathetic nerves. The DNA content of the segments was determined with the fluorometric assay of Labarca and Paigen (15) using calf thymus DNA as internal standard. This method exploits the enhancement by DNA of the fluorescence of bisbenzimidazole (Hoechst 33258). The NE content of the preparations was determined after extraction by high-performance liquid chromatography with fluorometric detection according to the method of Van der Hoorn (32a). The quantity of NE (pg) determined in the preparations was divided by the length of the vessel segments (mm) or by the DNA content of the vessel segments.

Solutions and drugs. The composition (mM) of the PSS was 119 NaCl, 4.7 KCl, 2.5 CaCl 2, 1.2 MgSO 4, 25 NaHCO 3, 1.2 KH 2PO 4, and 5.5 glucose. In high-K + solution, all NaCl was replaced by an equimolar amount of KCl. Drugs used were pentobarbital sodium (Sanofi, Bordeaux, France), Hoechst 33258 (Biochem SC, Brugge, Belgium), tetrodotoxin, NE, AVP, cocaine, ACh, SNP, calf thymus DNA, and bovine serum albumin from Sigma Chemical (St. Louis, MO). All drugs were dissolved in distilled water.

Data analysis. In all experiments, n equals the number of rats. Results are shown as means ± SE. Statistical significance of differences was evaluated using two-way analysis of variance (ANOVA) followed by post hoc comparisons with Fisher's least significant difference test. A P value of <0.05 was considered significant.

RESULTS

Vessel structure. After equilibration at 100 mmHg in vitro, lumen diameter was larger in HF arteries and smaller in LF arteries than in Sham (Table 1). Circumferential media stress did not, however, differ between experimental groups (Table 1). This is due to equivalent changes in media thickness accompanying the changes in lumen diameter. Media thickness (µm) averaged 15.7 ± 2.1 and 5.7 ± 0.6 in HF and LF, respectively. Both are significantly different from media thickness in Sham (10.0 ± 1.6 µm). Removal of extracellular calcium or administration of 100 µM SNP did not significantly modify the diameter in any of the vessel types (not shown), indicating the absence of basal vasmotor tone.

Constrictor reactivity. High potassium (125 mM K +) induced constriction in all vessels. In HF, the absolute constriction (µm) induced by 125 mM K + was significantly larger than in Sham, but the accompanying reduction in wall stress was comparable (Table 1). In LF, constrictor responses to 125 mM K + were drastically reduced (Table 1).

The maximal vasoconstriction induced by NE was larger in HF than Sham, but again the accompanying change in wall stress was comparable (Table 1). In LF, the maximal diameter and wall stress responses to NE were reduced (Fig. 1 and Table 1). The sensitivity to the constrictor effect of the catecholamine did not differ between vessel types (Fig. 1). The EC 50 averaged 0.63 ± 0.11, 0.85 ± 0.21, and 0.72 ± 0.10 µM in HF, LF, and Sham, respectively. In LF, the constrictor response elicited by the combination of 125 mM K + and 10 µM NE was also significantly reduced compared with Sham (Table 1). In contrast, diameter changes elicited by AVP were increased in HF and reduced in LF compared with Sham, but maximal wall stress responses to the peptide did not differ between experimental groups (Fig. 1 and Table 1). Also, the sensitivity to this peptide did not differ among HF, LF, and Sham (Fig. 1).

NE content per unit vessel length was comparable in HF and Sham and was reduced in LF (Table 2). When catecholamine content was expressed relative to the DNA content of the vessels, it was smaller in both HF and LF than in Sham (Table 2).

Electrical field stimulation (1–16 Hz) induced constrictions in all vessels. Responses to 16-Hz nerve stimulation were significantly larger in HF than Sham when both the reductions in diameter and in wall stress were considered (Table 1). Neurogenic responses were invariably smaller in LF than in Sham (Table 1).

Agenon- and flow-induced dilatations. All vessels dilated in response to SNP (0.01–100 µM) during preconstriction with 100 nM AVP (Fig. 2). Maximal responses to this direct vasodilator substance, expressed as a percentage of the preconstriction, did not differ among experimental groups. Sensitivity to the agent was comparable in HF (EC 50 = 0.074 ± 0.009 µM) and Sham (EC 50 = 0.074 ± 0.007 µM) but was more than 1 log unit smaller in LF (EC 50 = 2.50 ± 0.87 µM, P < 0.001) than Sham (Fig. 2).

Table 1. Constrictor responses in mesenteric small arteries exposed to HF or LF for 4 wk in comparison to vessels from Sham rats

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<tr>
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<th>Sham</th>
<th>HF</th>
<th>LF</th>
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<tr>
<td>Basal</td>
<td>413 ± 11</td>
<td>520 ± 10*</td>
<td>240 ± 16†</td>
</tr>
<tr>
<td>125 mM K +</td>
<td>-338 ± 15</td>
<td>-450 ± 11†</td>
<td>-65 ± 11†</td>
</tr>
<tr>
<td>10 µM NE</td>
<td>-285 ± 12</td>
<td>-383 ± 24†</td>
<td>-50 ± 5†</td>
</tr>
<tr>
<td>K + and NE</td>
<td>-366 ± 12</td>
<td>-465 ± 10†</td>
<td>-133 ± 26†</td>
</tr>
<tr>
<td>100 nM AVP</td>
<td>-280 ± 12</td>
<td>-437 ± 19†</td>
<td>-135 ± 9†</td>
</tr>
<tr>
<td>16 Hz EFS</td>
<td>-107 ± 9</td>
<td>-186 ± 19†</td>
<td>-38 ± 12†</td>
</tr>
<tr>
<td>Media stress, N/m²</td>
<td>15</td>
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**Sham** HF LF

| Basal    | 100  | 100  | 100  |
| 125 mM K + | -82 ± 2  | -87 ± 1  | -27 ± 5† |
| 10 µM NE  | -69 ± 1  | -73 ± 3  | -23 ± 3† |
| K + and NE | -88 ± 1  | -94 ± 4  | -54 ± 10† |
| 100 nM AVP | -88 ± 2  | -82 ± 4† | -61 ± 7 |
| 16 Hz EFS | -24 ± 2  | -36 ± 4† | -14 ± 3† |
| Media stress, N/m² | 28 ± 2  | 271 ± 35  | 283 ± 15  |

**Basal conditions and changes induced by high-potassium solution (K ++), norepinephrine (NE), arginine vasopressin (AVP), and electrical field stimulation (EFS) are shown as means ± SE; n = 7 rats. Sham, sham-operated rats; HF, high blood flow; LF, low blood flow. *Difference from Sham is statistically significant at *P < 0.05, †P < 0.01, and ‡P < 0.001, respectively.**
Endothelium-dependent vasodilator responses were also modified. ACh (10 nM to 10 µM) induced dilatation of vessels preconstricted with NE (Fig. 2). The maximal effect did not differ among groups. The sensitivity to the agent was comparable in HF (EC50: 52 ± 7 nM) and Sham (EC50: 63 ± 8 nM) but was markedly smaller in LF (EC50: 800 ± 17 nM, P < 0.001).

Intraluminal flow (100–1,000 µl/min) induced dilatation of vessels preconstricted with 10 µM NE (Fig. 2). Maximal flow-induced dilatation, expressed as a percentage of NE constriction, did not differ significantly from Sham (40 ± 5%) in either HF (30 ± 5%) or LF (50 ± 6%) but was larger in LF compared with HF (P < 0.01). Observations during stepwise increases in perfusate flow were used to calculate wall shear stress. In Fig. 3 the relationship between this parameter and flow or arterial diameter is illustrated. In Sham, flows between 100 and 700 µl/min modified diameter to maintain shear stress at 63 ± 10 dyn/cm². In HF, the wall shear stress–diameter curve was shifted to the left; i.e., the maintained shear stress value was reduced to 48 ± 7 dyn/cm² (P < 0.01). In LF, calculated wall shear stress increased with increasing perfusate flow (Fig. 3).

DISCUSSION

It has become increasingly clear that chronically maintained or intermittent elevation of blood flow leads to increases in the lumen diameter and wall mass of arteries (22, 30, 31, 34) and to alterations in the function of their endothelium (13, 18, 33). We evaluated whether changes also occur after long-term hyperperfusion and whether the arterial smooth muscle, endothelium, and perivascular nerves are involved in modified arterial reactivity. Arterial responses to increased flow are important for the collateral circulation in ischemia (5, 24), circulatory adjustments during endurance training (13), and the uterine circulation during pregnancy (20). Responses to reduced blood flow are likely to develop downstream from stenoses and in the uterine circulation postpartum. Structural changes in response to altered flow involve changes in the volume and number of the medial smooth muscle cells (22, 31). It is, in our opinion, an interesting possibility that these could be accompanied by quantitative and qualitative changes in excitation-contraction coupling (21). Whereas the endothelium (17, 30, 31), the media (22, 30, 31), and to some extent the adventitia (22) have been addressed with respect to flow-induced arterial remodeling, little attention has been given to the perivascular autonomic innervation. Distension of arterial vessels by elevated pressure or through dilatation has, however, been shown to activate nerves in the aortic arch and carotid bodies as part of the baroreflex (2) and in the cranial arteries during the course of a migraine attack (9). We hypothesize that flow-induced dilatation, which may precede flow-induced arterial remodeling (12, 30, 34), interferes with perivascular nervous function as well.

We used an experimental animal model comparable to the one recently developed by Unthank et al. (31, 32) in which side branches of the superior mesenteric artery are ligated in rats in order to elevate flow through the unobstructed branches. In our model we ligated every other branch in an attempt to induce comparable increases and decreases in flow in parallel vessels. Although no collateral vessels were formed around the ligations, a small but significant flow persisted in the obstructed vessels. This is most likely due to the presence of small branches proximal from the ligation that perfuse the mesentery. Although mesenteric blood flow changes considerably during the day, the persistent hyperperfusion and hypoperfusion of individual vessels that we created must be regarded as artificial. Especially the 90% flow reduction is likely nonphysiological. It may, however, provide information about the type of alterations resulting from chronic hypoperfusion in general. Part of these were monitored by recording vasoconstrictor and vasodilator responses to agonists. This pharmacological evaluation was not intended to represent the normal physiological control

Table 2. NE content in rat mesenteric small arteries exposed to HF or LF for 4 wk in comparison to vessels of Sham rats

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<tr>
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<th>Sham</th>
<th>HF</th>
<th>LF</th>
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<tr>
<td>NE, pg/mm</td>
<td>260±30</td>
<td>228±34</td>
<td>126±21*</td>
</tr>
<tr>
<td>NE, pg/mg DNA</td>
<td>10.2±1.4</td>
<td>4.0±0.5*</td>
<td>4.9±1.1*</td>
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Values are shown as means ± SE; n = 7 rats. *Difference from Sham is statistically significant (P < 0.05).
of vasomotor tone, since basal vasoconstriction is rather small in first-order mesenteric artery side branches of the rat.

At 4 wk after the surgical intervention, lumen diameter and media thickness were increased in the HF vessels and reduced in the LF vessels as previously reported (22). In both conditions, resting circumferential wall stress was, however, comparable to that in vessels from sham-operated rats. Thus, within the experimental period, outward hypertrophic and inward hypotrophic remodeling developed in order to normalize circumferential wall stress and presumably wall shear stress (22).

The hypertrophy of the hyperperfused vessels has been attributed to hyperplasia of vascular wall cells, including arterial smooth muscle cells (22, 31). In a recent preliminary three-dimensional dissector analysis of fixed vessels, we directly demonstrated hyperplasia in the absence of a modified cellular volume. We found that this was accompanied by altered arterial reactivity. For every type of stimulus that we used, maximal constrictions were increased compared with control vessels. The accompanying changes in media stress were, however, with the exception of periartrial nerve stimulation, not modified. The hyperreactivity can thus be attributed to a larger muscle mass. Part of the new muscle seems to be laid down in series with the original contractile units because the maximal agonist-induced diameter reductions were significantly increased (19). Sensitivity to NE, AVP, and SNP was not modified in the widened hypertrophic vessels, suggesting that no major qualitative changes had developed in the excitation-contraction coupling. NE content was not modified per unit vessel length but was reduced when normalized to tissue DNA content. Wall hypertrophy due to hyperplasia had thus “diluted” the density of the perivascular sympathetic nerves. Yet, constrictor responses to stimulation of these nerves were, if anything, increased in the chronically hyperperfused arteries. Apparently, communication between arterial smooth muscle cells was maintained (or restored) in this hyperplastic setting.

Because neither sensitivity nor maximal responses to the dilator action of SNP or ACh were modified, no major alteration developed in agonist-induced endothelium-dependent or -independent dilatation of hyperperfused arteries. Coronary collateral arteries, established in a canine model of progressive coronary artery occlusion, have also been shown to display unaltered acetylcholine-induced responses (7). Acute dilator responses of rat small arteries to flow have previously been attributed to shear stress-induced release of NO and prostaglandins by the endothelium (14, 23). In chronically hyperperfused mesenteric arteries, in vitro dilator
responses to flow were somewhat reduced. Compared with controls, these vessels, however, maintained a significantly lower level of wall shear stress during exposure to flow. A similar change has been observed in gracilis muscle arteries of rats after 3 wk of daily exercise (13). These results, combined with our observations in rat mesenteric arteries that adapted to elevated blood flow, indicate 1) outward hypertrophic remodeling of the vessel wall; 2) normal contractile reactivity of the arterial smooth muscle, which in combination with hypertrophy leads to exaggerated vasoconstriction to exogenous and neurogenic stimuli; and 3) normal relaxing responses of the arterial smooth muscle to directly acting and endothelium-dependent agents. The sensitivity of the endothelium of these HF vessels to wall shear stress may, however, be upregulated.

Remarkably different findings were obtained in vessels that had been exposed for 4 wk to reduced blood flow. Maximal constrictor responses to NE and high potassium were drastically reduced. This was not solely due to reduced muscle mass as 1) maximal media stress responses were reduced as well and 2) maximal wall stress responses to AVP were not reduced. The most likely explanation for this observation is that a selective modification of the excitation-contraction coupling developed during the exposure to reduced flow. It is not likely to involve altered \( \beta \)- or \( \alpha_2 \)-adrenergic responsiveness, because the presence of propranolol and yohimbine did not modify the hyporesponsiveness (unpublished observations). We recently described that also in models of heart failure (28) and viral sepsis (8), rat mesenteric artery responses to \( \alpha_1 \)-adrenergic stimulation were reduced but contractile response to AVP was not modified. We suggested that in these conditions a selective modification of pertussis toxin-sensitive G proteins could be involved. Whether this could also be the case following chronic blood flow reduction remains to be established. In line with the reduced responses of the LF vessels to exogenous NE, their constrictor responses to autonomic nerve stimulation were also reduced. Besides postjunctional aspects, this may also involve a prejunctional component because the NE content of these vessels was reduced when normalized to both length and DNA content. A structural change of the nerves may have accompanied the adaptations of the vessels to reduced flow. Immunohistochemical and morphometric analyses of the perivascular innervation will be required to prove that autonomic nerve density is actually reduced in this setting.

The three mechanistically related dilator responses that we studied were markedly reduced in the LF vessels. In the case of both SNP and ACh, sensitivity was reduced by a log unit or more. Near-complete dilatation could, however, still be obtained. This suggests impaired guanosine 3',5'-cyclic monophosphate production or reduced sensitivity to the relaxing effect of this second messenger in the arterial smooth muscle. This may have entailed reduced dilator responses to shear stress as well. An additional contribution of reduced sensitivity of the endothelium to wall shear stress cannot be excluded.

In general, our observations concerning endothelium-dependent and flow-induced dilatation in vessels chronically exposed to elevated or reduced flow are in line with the concept that wall shear stress modulates arterial endothelial function on both an acute (14, 23, 25) and a chronic basis (12, 17, 30, 33, 34). It seems, however, that chronic influences of blood flow include modulation of 1) the sensitivity of the endothelium to shear stress (13 and this study), 2) expression of the endothelial NO synthase (27), 3) endothelial production of NO (27, 29, 33), and 4) responsiveness of the arterial smooth muscle to NO (this study). Moreover, adaptation to elevated blood flow and to reduced blood flow seems to affect different aspects. In future experiments, it may be of interest to evaluate the effects of endothelium removal and pharmacological modulation of NO synthase activity with respect to structural and functional consequences of chronic changes in blood flow.

In summary, we evaluated in vitro vasomotor responses in rat mesenteric small arteries that had remodeled in response to elevated blood flow or to reduced blood flow. In the HF vessels, increased mass of the otherwise normal arterial smooth muscle and increased sensitivity of the endothelium to wall shear stress can account for most of our observations, including increased neurogenic vasoconstrictor responses and maintenance of a lower wall shear stress in vitro. In the LF vessels, on the other hand, besides hypotrophy of the wall, a reduced responsiveness of the arterial smooth muscle to certain vasoconstrictor stimuli and to NO was observed along with a reduction of sympathetic neurotransmitter content.

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