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High Intravascular Pressure Attenuates Vascular Dilation Responses of Small Mesenteric Arteries in the Rat

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Background Alterations in intravascular pressure can affect vascular function and the morphological properties of arteries. Although it has been shown that a transient elevation of blood pressure impairs endothelium-dependent dilation in small arteries, the vascular dilation responses during high intravascular pressure have not yet been investigated.

Methods and Results Using pressure myography, the endothelium-dependent and -independent dilation responses of small mesenteric arteries of rats were examined under 3 different intravascular pressure conditions (50, 80 and 120 mmHg). Endothelium-dependent dilation was evaluated by measuring vasodilator responses to increasing doses of acetylcholine (ACh, 10^{-9} to 10^{-4} mol/L) or increases in intraluminal flow (7–36 μl/min). Endothelium-independent vasodilator function was examined by using sodium nitroprusside (SNP, 10^{-9} to 10^{-4} mol/L). Flow-mediated dilation was significantly attenuated at 120 mmHg whereas ACh-induced dilation was progressively decreased with increases in intravascular pressure (at 80 and 120 mmHg). Significant attenuation in the vasodilator response to SNP was also observed at 80 and 120 mmHg.

Conclusion Endothelium-dependent and-independent dilation responses are progressively attenuated with acute increases in intravascular pressure. (*Circ J* 2008; **72:** 482–486)

Key Words: Arteries; Blood pressure; Vasodilation

Previous studies have demonstrated that hypertension and cardiovascular diseases are associated with functional and morphological changes in arteries.[1–3] In hypertension, the small arteries and arterioles that determine peripheral resistance undergo changes such as increased reactivity to contractile agents, impaired endothelial function, and vascular smooth muscle growth.[4,5] Although it is unclear whether these are primary or secondary abnormalities, it is plausible that the increased intravascular pressure itself has some deleterious effect on vascular functional and/or morphological properties, and it has been proposed that such functional and morphological changes in arteries could be prevented by normalizing intravascular pressure.[6] Thus, independent of origin, high intravascular pressure can directly induce structural and functional modifications of the arterial wall.

Blood vessels are continuously exposed to biomechanical forces, such as consist of shear stress induced by the movement of blood through the vessel lumen and stretch determined by luminal pressure and compliance. Chronic alterations in these forces could be important in vascular remodeling induced by both physiological (ie, exercise training) and pathological (ie, hypertension) conditions.[7] However, the variations in these mechanical forces can occur acutely and transiently in vivo. It is well documented that stretching of the arterial wall by increased transmural pressure alters the mechanical properties of vessels, as well as triggering various intracellular signaling pathways.[8–10] Thus it is possible that the vascular responsiveness of arteries also changes under conditions of high intraluminal pressure.

However, to our knowledge, the vascular responses of vessels from normotensive subjects under high intraluminal pressure conditions have not yet been investigated. Rather, in previous studies, vascular responses to shear stress or agonists have been assessed at basal intraluminal pressures after a defined period of high intraluminal pressure.[11,12] Those myography studies using pre-pressurized vessels from normotensive subjects have demonstrated that endothelium-dependent dilation responses are directly attenuated by transient elevations in intravascular pressure,[11,12] while endothelium-independent vasorelaxation was found to be unaffected.[12] Because the vessel responses were examined after decreasing pressure to the original basal conditions, the mechanical effect of sustained high intravascular pressure on the vascular responses was not explored. Thus, the aim of this study was to investigate the vascular dilation responses of small mesenteric arteries (SMA) in rats at high intra-arterial pressure conditions. For this purpose, we examined the endothelium-dependent and -independent dilation responses under 3 different intravascular pressure conditions (ie, 50, 80 and 120 mmHg) using a pressure myography method.

Methods

Animals

Thirty adult male Wistar rats, 14 weeks old and weighing 300–320 g, were used in the present study. The animals

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were housed at 23±2°C on a 12:12 h light–dark cycle and had free access to standard rat chow and drinking water. The experimental protocol was approved by the Animal Care and Usage Committee of Akdeniz University and was in accordance with the Declaration of Helsinki and International Association for the Study of Pain (IASP) guidelines.

Isolation of Mesenteric Resistance Arteries

Rats were anesthetized with an intraperitoneal injection of thiopental sodium (80 mg/kg body weight) and the mesenteric bed was removed and placed in a dissecting dish filled with MOPS-buffered physiological saline solution (PSS) containing (in mol/L) 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 25.0 MOPS, at pH 7.4. Under a dissecting microscope (SZ61, OLYMPUS, Tokyo, Japan), A second-order branch of the mesenteric artery was isolated from the mesenteric vascular bed and carefully cleaned of surrounding tissue. One or 2 arterial segments were isolated from each animal and transferred to a vessel chamber (CH/1, Living Systems, Inc, Burlington, VT, USA) containing 2 glass micropipettes.

After cannulation of the vessel at 1 end, the perfusion pressure was raised to 20 mmHg to clear the clotted blood from the lumen, and then the other end of the vessel was mounted on the distal pipette and tied with a surgical nylon suture. Each micropipette was attached to a computer-controlled roller pump via tubing equipped with an in-line pressure transducer so that the inflow and outflow pressures for the arterial segment could be monitored. Both inflow and outflow micropipettes were connected with silicone tubing to a pressure-servo pump system in which pressure and flow could be controlled independently (Living Systems). The flow chamber with the arterial segment preparation was placed on an inverted microscope (Eclipse TS100, Nikon, Tokyo, Japan) equipped with a charge-coupled device camera (XC73CE, Sony, Tokyo, Japan). The camera was connected to a video dimension-analysis system (model V94, Living Systems), which enabled continuous measurement of the vessel diameter. The mean pressure, pressure gradient, fluid flow rate through the arterial segment, and the internal vessel diameter were continuously recorded via a data acquisition system (MP 100A-CE; BIOPAC Systems, CA, USA) connected to a personal computer. The arteries were perfused with MOPS-PSS supplemented with albumin (1 g/100 ml) and the axial length of the arterial segment was adjusted by positioning the cannula until the vascular walls were parallel without obvious stretch. Intraluminal pressure was set to 50 mmHg with the servo-controlled pump and the arterial segment was allowed to equilibrate at this pressure for 60 min at 37°C under no-flow conditions. Three separate series of experiments were performed with the arterial segments to determine the dilation responses to acetylcholine (ACh), flow and sodium nitroprusside (SNP). Preliminary experiments had showed that repetition of the ACh, SNP or flow-diameter curves in the absence of high intraluminal pressure led to similar responses, thus ruling out any time-related effects.

Endothelium-Dependent Dilation

ACh-Induced Dilation After the equilibration period the pressurized arteries were preconstricted to 55–60% of their original diameter by adding phenylephrine to the bath (10^{-7}–10^{-5} mol/L), after which increasing concentrations of ACh (10^{-9}–10^{-4} mol/L) were added (n=11). The vessels were then washed, the perfusion pressure was increased to 80 mmHg, and the vessels were incubated at this pressure for 30 min. At the end of the incubation period, the vessels were again preconstricted with phenylephrine and the responses to ACh were obtained at 80 mmHg. After the last washout, the pressure was raised to 120 mmHg for another 30 min and the dose–response curve of ACh was obtained at this pressure.

Flow-Mediated Dilation (FMD) FMD was assessed at a series of fluid flow rates between 7 and 36 μl/min while the intraluminal pressure was kept constant by the pressure servo-control system (n=13); the speed of the upstream roller pump was manually adjusted to generate the required flow rate. Each flow rate was maintained for 5 min to allow the vessel to reach a steady diameter. After obtaining the FMD responses of vessels preconstricted with phenylephrine at 50 mmHg, the pressure was raised to 80 mmHg for 30 min and FMD was assessed at that pressure. After the last washout, the pressure was raised to 120 mmHg for another 30 min and the procedure to assess FMD was repeated.

Endothelium-Independent Dilation

Endothelium-independent dilation was assessed by adding increasing doses of SNP to the bath solution over the range of 10^{-9}–10^{-4} mol/L (n=10). The relaxation responses to SNP were obtained at the 3 different intraluminal pressure levels (50, 80 and 120 mmHg) using the same procedure as before.

Passive Diameter At the end of each experiment, vessels were incubated with Ca^{++}-free PSS to determine passive diameters at 50, 80 and 120 mmHg intraluminal pressure.

Statistical Analysis

All values are given as means ± SE. The responses to ACh, SNP and flow are expressed as percentages of the reversal of the phenylephrine-induced constriction. The concentration of drug (ACh or SNP) that produced one-half of the maximal response, termed EC_{50}, was estimated using nonlinear regression analysis (GraphPad Software Inc, San Diego, CA, USA). The sensitivity to each agonist was expressed as $pD_2 = -\log EC_{50}$. ANOVA for repeated measurements followed by the Bonferroni test were used for comparison of the response curves. A p-value <0.05 was considered significant.

Results

Reproducibility of Responses at Low Pressure

In a series of preliminary experiments, there were no significant differences for a given segment in the repeated response curves to flow, ACh or SNP at 50 mmHg intraluminal pressure (data not shown).

Characteristics of the Isolated Arteries

The respective maximal passive diameters of the SMA used in the present study were 211±8, 225±9 and 234±10 μm at 50, 80 and 120 mmHg. The maximal diameter significantly increased at 80 and 120 mmHg compared with 50 mmHg ($p<0.01$ and $p<0.001$, respectively). The arteriolar tone (% of passive diameter) was similar under all pressure conditions (data not shown).

Endothelium-Dependent Dilation

ACh-Induced Dilation Fig 1 illustrates the responses to

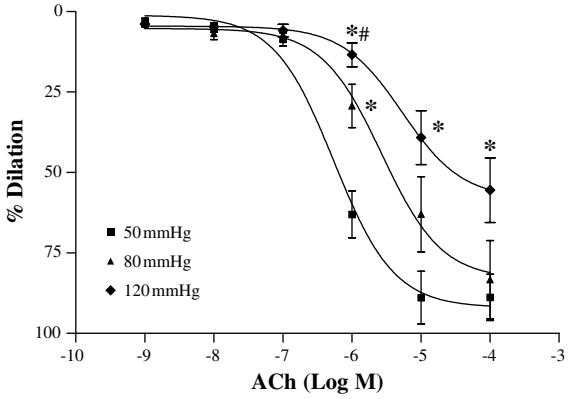

Fig 1. Acetylcholine (ACh)-induced dilation responses of rat small mesenteric arteries during progressive increases of intraluminal pressure. Values are means ± SE. *p<0.05, difference from 50 mmHg; #p< 0.05, difference from 80 mmHg.

Table 1 pD2 (-Log EC50) Values for ACh- and SNP-Induced Relaxation in Rat Small Mesenteric Arteries at 50, 80 and 120 mmHg Intraluminal Pressure

	n	*50 mmHg*	*80 mmHg*	*120 mmHg*
ACh	*11*	*6.261±0.12*	*5.571±0.22**	*5.273±0.25*#*
SNP	*10*	*7.109±0.39*	*6.213±0.35**	*5.925±0.50*#*

Values are means ± SE.
**p<0.001, difference from 50 mmHg; #p<0.001, difference from 80 mmHg.*
ACh, acetylcholine; SNP, sodium Nitroprusside.

ACh at the 3 different intraluminal pressures. There was a right shift of the concentration–response curve with the increase in intraluminal pressure. The dilation response at 10^{-6} mol/L ACh was significantly and progressively attenuated at 80 and 120 mmHg ($p<0.05$). In addition, there was a significantly reduced dilation response at 10^{-5} and 10^{-4} mol/L ACh for 120 mmHg compared with 50 mmHg ($p<0.05$). Accordingly, the calculated sensitivity to ACh (ie, $-\log EC_{50}$) was significantly decreased at both 80 and 120 mmHg ($p<0.001$) compared with 50 mmHg (Table 1).

FMD Fig 2 demonstrates the FMD response of the SMA to increases in perfusate flow at 50, 80 and 120 mmHg intraluminal pressures. Although there was decreased dilation at 80 and 120 mmHg, significant effects vs 50 mmHg were only seen at 120 mmHg and an intermediate flow rate (26 μl/min, $p<0.05$).

Endothelium-Independent Dilation

Fig 3 illustrates the vasodilator response of the SMA to SNP, from which it is clear that the response was reduced with increases of intravascular pressure; that is, a progressive decreased at 80 and 120 mmHg compared with 50 mmHg over the range of 10^{-8}–10^{-5} mol/L SNP ($p<0.05$). The dilation response to highest dose of SNP (10^{-4} mol/L) was significantly attenuated only at 120 mmHg compared with 50 or 80 mmHg ($p<0.05$). The calculated sensitivity to SNP (ie, $-\log EC_{50}$) gradually and significantly decreased as the intraluminal pressure increased to 80 or 120 mmHg (Table 1, $p<0.001$).

Discussion

The results of this study indicate that increases in intra-

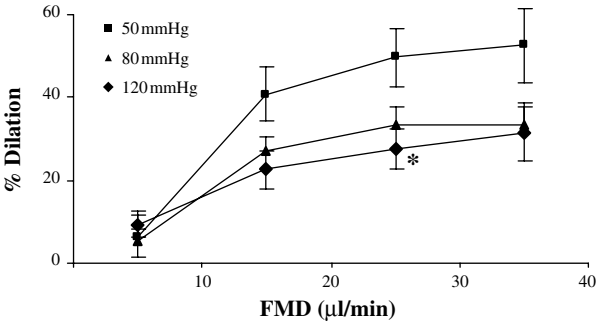

Fig 2. Flow-mediated dilation (FMD) responses of rat small mesenteric arteries during progressive increases of intraluminal pressure. Values are means ± SE. *p<0.05, difference from 50 mmHg.

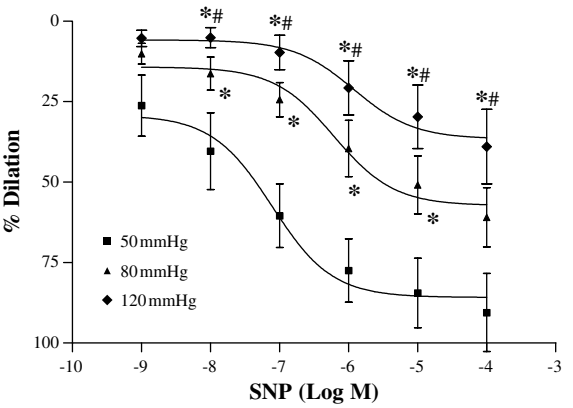

Fig 3. Sodium nitroprusside (SNP)-induced dilation responses of rat small mesenteric arteries during progressive increases of intraluminal pressure. Values are means ± SE. *p<0.05, difference from 50 mmHg; #p<0.05, difference from 80 mmHg.

arterial pressure significantly impair both the endothelium-dependent and -independent dilation responses of SMA from normotensive Wistar rats. Although this was a non-mechanistic study, it is the first to investigate the responsiveness of small arteries from normotensive rats under high intraluminal pressures.

Elevated blood pressure is a factor that not only emerges during hypertension, but also appears during exercise training or mental stress, is the major determinant of blood vessel stretch, and affects all cell types of the vessel wall. It is well known that alterations in stretch, as well as shear stress, instigate important adaptive alterations by modifying the morphology and function of vessels[7,13] Acute changes in stretch or shear stress correlate with transient adjustments in vessel diameter, mediated through release of vasoactive agonists or changes in myogenic tone. In addition, pulsatile change in transmural pressure may be involved in the autoregulation of flow, especially in some tissues such as myocardium![14] On the other hand, chronically altered mechanical forces usually induce adaptive alterations of vessel wall shape and composition, which is termed vascular remodeling![15–17] It has been shown that acute and transient high intraluminal pressure cause endothelial dysfunction and impaired endothelium-dependent vascular dilation![11,12] However, those prior studies did not evaluate the vascular dilation responses during high intraluminal pressure conditions. Rather, the intraluminal pressure was increased to a high level for 30 or 60 min and the responses of vessels were

evaluated at normotensive conditions after this incubation period. Thus the possible effects of tensile stress induced by high pressure on vascular responsiveness were ruled out.

Our results indicate that endothelium-dependent dilation of rat SMA is attenuated during exposure to intra-luminal pressures of 80 and 120 mmHg: both ACh- and flow-induced dilation responses were reduced. In addition, the calculated sensitivity to ACh was significantly decreased with increases in intraluminal pressure (Table 1). Note that the results of our preliminary experiments at 50 mmHg showed that none of ACh-, flow- or SNP-induced dilation was impaired over time, thereby excluding artifacts caused by temporal deterioration of the vessels. These results thus support the finding that endothelial function is impaired during high intra-arterial pressure and, based upon previous studies, indicate that this impairment persists at normotensive conditions for at least a short period after the pressure increase[10,11]

Oxidative stress induced by several factors may lead to endothelial dysfunction[18,19] One of the mechanisms underlying the reduction of endothelium-dependent dilation induced by ACh and/or flow may involve increased free radical production because of the high intravascular pressure. Both cyclic and sustained stretch has been shown to increase free radical production by vascular cells, primarily via activation of the NADPH-oxidase enzyme system[15–17,19] Increased oxidative stress in response to stretch may alter endothelium-dependent dilation by reducing the bioavailability of nitric oxide (NO). Huang et al have shown that transient high intra-arteriolar pressure induces superoxide release and reduces the NO-mediated portion of FMD in rat gracilis muscle arterioles[11] In the present study, we assessed endothelium-dependent dilation under sustained high intravascular pressure conditions that results in stretch of both vascular endothelial and smooth muscle cells. Therefore, in addition to oxidative stress, mechanical effects may activate some intracellular signaling pathways that contribute to impaired dilation responses. In fact, studies of cultured vascular cells have shown that mechanical stimulation is able to activate intracellular signaling systems that were followed by specific cellular responses[17,20–22] Other possible explanations for our results include increased production of vasoconstrictor prostaglandins and/or endothelial contractile factors from vascular endothelial cells stimulated by elevated transmural pressure[22–24]

We also assessed the endothelium-independent dilation of SMA by using SNP: dilation responses to increasing doses of SNP were significantly reduced at high pressures and hence the sensitivity to SNP (ie, EC_{50}) was also reduced. These results suggest that sustained high transmural pressure reduces the ability of vascular smooth muscle to dilate in response to nitro-vasodilators. In contrast, Paniagua et al showed that transient hypertension did not affect the response to SNP in isolated small human arteries[12] The reasons for this discrepancy are unclear at the moment, but may depend on the species and/or methods used. Those authors used human rather than rat vessels and, in contrast to us, obtained their responses to SNP after a transient increase in intraluminal pressure.

It must be noted that our experimental design did not allow us to evaluate the mechanistic relationship between high pressure and impaired dilation responses. However, the impairment of smooth muscle cell dilation response as a consequence of high intraluminal pressure could be involved in the pathophysiology of hypertension in which vascular smooth muscle cells are permanently exposed to stretching because of the forces generated by blood pressure. Pressure leads to strain on the vessel wall and longitudinal stretch of the smooth muscle cells. Meanwhile, nonselective cation channels (ie, K^+, Na^+, Ca^{++}) activation and reactive oxygen species production are also triggered by high pressure[15,17,19] We speculate that these alterations induced by high intravascular pressure contributed to the impaired endothelium-independent dilation observed in the present study.

In conclusion, the results of this study indicate that high intravascular pressure impairs both endothelium-dependent and -independent dilation of rat SAM; the effects were characterized by attenuated vasodilator responses to flow, ACh and SNP. Although our results do not provide mechanistic information, the findings may be important for understanding the effects of high intraluminal pressure on the responsiveness of small arteries. Further studies are required to determine the underlying mechanisms of reduced vascular dilation responses during high intraluminal pressure.

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