AJH 1992; 5:542-547

Intraluminal Pressure Modulates Vascular Contractility of Perfused Mesenteric Resistance Arteries

Altered Response in Hypertension

Michael A. Thiel, Andreas H. Bock, Fritz R. Bühler, and Thomas F. Lüscher

Intraluminal pressure may affect vascular contractility in both normotension and hypertension. To test this hypothesis, we studied mesenteric resistance arteries from normotensive humans as well as normotensive (WKY) and spontaneously hypertensive (SHR) rats (internal diameter 214 ± 27, 201 ± 6, and 172 ± 6 μm, mean ± SEM at 10 mm Hg). Vessels were mounted on glass cannulas and perfused in organ chambers filled with buffer solution at intraluminal pressures of 10 to 120 mm Hg; vasomotion was measured using a video dimension analyzer. Under baseline conditions (10 mm Hg), wall thickness was 36 ± 4 μm in humans, 32 ± 4 μm in WKY, and 47 ± 2 μm in SHR ($P < .001$). With increasing pressure, the diameter of human vessels increased up to 25 mm Hg and remained constant at higher pressures. In contrast, resistance arteries of normotensive and hypertensive rats exhibited an almost linear increase in diameter over the whole pressure range. In SHR, the pressure-diameter relationship was much flatter than that of WKY, indicating reduced compliance. In human arteries, the contraction to KCl was maximal at 25 mm Hg and averaged 40 ± 6%. Both above and below 25 mm Hg, the response declined to a minimum of 17 ± 2% at 120 mm Hg ($P < .01$). Similar results were obtained in WKY rats. In contrast, the contractile response in SHR remained maximal over the entire pressure range studied (65 ± 5%). Thus, intraluminal pressure profoundly affects vascular reactivity of resistance arteries; low pressure augments and high pressure reduces the contractile response in normotensive human and rat resistance arteries, whereas this pressure-dependent modulation of vascular reactivity is lost in the SHR. Am J Hypertens 1992;5:542-547

KEY WORDS: **Microcirculation, perfusion, pressure.**

In vivo arteries are exposed to various intraluminal pressures and flow rates. These mechanical forces have profound effects on the function of vascular smooth muscle cells and the endothelium.[1-5] In hypertension, these forces are increased and may importantly contribute to the altered vascular reactivity associated with the disease.[6]

In hypertension research, the influences of autacoids, hormones, and endothelium-derived substances on vascular reactivity have been measured as changes in the isometric tension of isolated rings of vascular smooth muscle, or as changes in intraluminal diameter of perfused arteries. However, the effects of basal tension or perfusion pressure on contractile responses have rarely been considered.

This study was designed to investigate whether and how intraluminal pressure affects the passive and active vascular properties of perfused mesenteric resistance ar-

Received October 29, 1990. Accepted April 28, 1992.

From the Department of Research, Laboratories of Vascular Research (MAT, TFL), Hypertension (FRB), Nephrology (AHB), and the Department of Medicine, Division of Cardiology (TFL), University Hospital, Basel, Switzerland.

This study was supported by grants from the Swiss National Research Foundation (no. 32-25468.88; Score-grant no. 3231-025150) and the Swiss Cardiology Foundation.

Address correspondence and reprint requests to Thomas F. Lüscher, MD, Department of Research, Laboratory of Vascular Research, University Hospital, CH-4031 Basel, Switzerland.

0895-7061/92/$5.00

teries of humans and the rat, and whether alterations of pressure-dependent vascular responses occur in hypertension.

MATERIALS AND METHODS

Sources of Vascular Tissue We obtained human mesenteric tissue during abdominal surgery in patients with normal blood pressure (≤ 140/90 mm Hg) and taking no antihypertensive drugs when admitted (four cases of cancer of the colon and three cases of abdominal adhesions).

Male, 20- to 28-week-old Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats were obtained from Madörin (Frenkendorf, Switzerland). We measured blood pressure in conscious rats with the tail cuff method and averaged 148 ± 3 mm Hg in Wistar-Kyoto and > 200 mm Hg in spontaneously hypertensive rats. The rats were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). Through an abdominal incision, we removed the mesentery and placed it into cold (4°C) modified Krebs-Ringer solution of the following composition (mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, Na_2Ca EDTA 0.026, glucose 11.1, vigorously bubbled with 95% O_2 and 5% CO_2. Small mesenteric resistance arteries (193 ± 10 μm, n = 19) were carefully dissected free from surrounding connective tissue under a dissection microscope and 3 to 5-mm-long segments were mounted on a perfusion system.

Experimental Setup Details of the perfusion setup have been previously described.[7,8] In brief, the proximal end of the arterial segment was slipped onto a microcannula (outer diameter 150 μm) and fixed by a surgical suture no. 11 (25 μm). The distal end of the vessel was sucked into the end of an efferent glass cannula. To apply a distal resistance, the inner diameter of the efferent cannula was slightly smaller than the inner diameter of the afferent cannula plus twice the thickness of the vessel wall. This allowed a pressure dependent perfusion rate of 0.2 to 0.9 mL/min with a calculated pressure drop along the vessel length of only 2 to 6 mm Hg. An almost constant lumen diameter observed over the whole vessel length indicated the absence of a considerable pressure drop caused by a hidden leak.

The arteries were perfused at a constant pressure with Krebs-Ringer solution containing 1% albumin from bovine serum. Perfusion pressure was measured directly at the proximal end of the arterial segment by a microcatheter connected to a Statham (Cleveland, OH) transducer system. Both afferent and efferent cannula were immersed in a chamber filled with circulating (37°C) Krebs-Ringer solution. This chamber was subsequently placed on a Leitz inverted microscope with an RCA television camera attached to the viewing tube. We continuously measured the inner diameter and wall thickness of the resistance arteries with a video dimension analyzer (Living Systems Instrumentation, Burlington, VT). Contractions were elicited by adding KCl to the extraluminally circulating Krebs-Ringer solution (100 mmol/L).[13]

Experimental Protocols After equilibrating the vessels for 1 h at a constant perfusion pressure (10 mm Hg), we started the experiment with a contraction at the same pressure. After washout, perfusion pressure was increased stepwise to 120 mm Hg, and the arteries were allowed to equilibrate again at each new pressure step for another 20 min before the next contraction. To ensure an intact contractility at the end of the whole experiment, an additional contraction was elicited at the pressure where the maximal response had been observed. We excluded experiments in which these contractions were not comparable to the previously recorded ones.

Calculations and Statistical Analysis Contractions are expressed as the percentage decrease of the initial inner diameter. Data are expressed as mean ± SEM; n refers to the number of patients or rats studied. For statistical analysis, Mann-Whitney test was used to compare vascular dimensions (Figure 1). To compare the effects of increasing intraluminal pressure on internal vascular diameter in WKY and SHR (Figure 2), a combined analysis of variance and covariance was used.[9] To compare contractility between various pressure levels (Figures 3 and 4), a two-way analysis of variance followed by the least significant difference test was used.

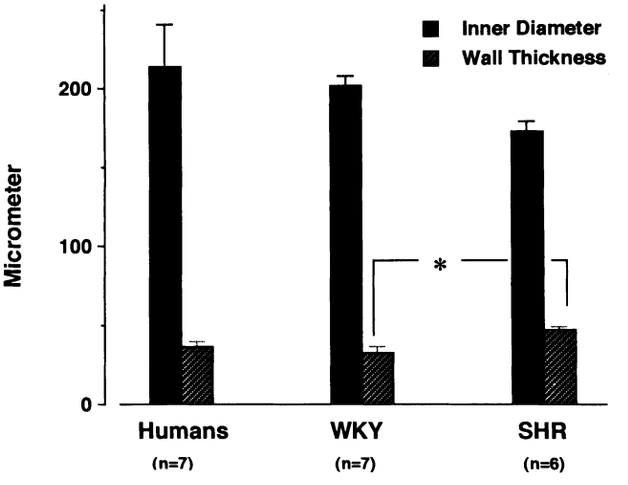

FIGURE 1. *Dimension of mesenteric resistance arteries obtained from normotensive humans, WKY, and SHR under baseline conditions (10 mm Hg).* *P < .001.

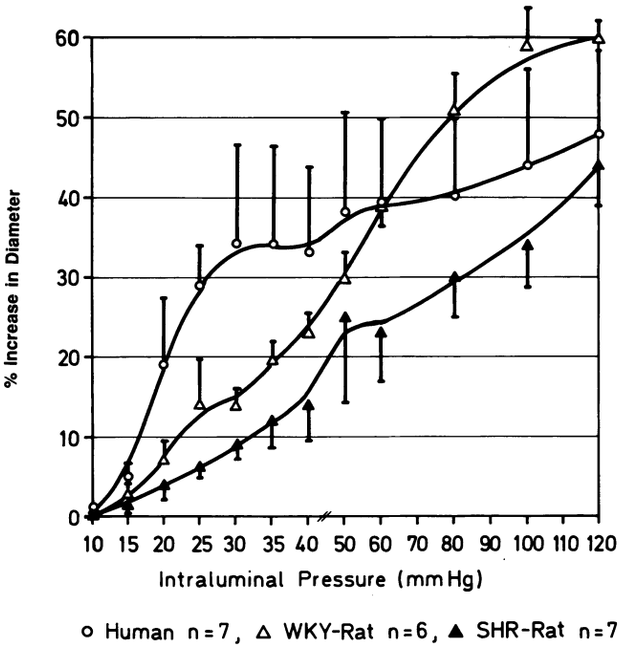

FIGURE 2. *Effects of intraluminal pressure on internal vascular diameters in mesenteric resistance arteries obtained from normotensive humans, WKY, and SHR. The increase in diameter in SHR arteries was significantly flatter compared with arteries from WKY (P < .001).*

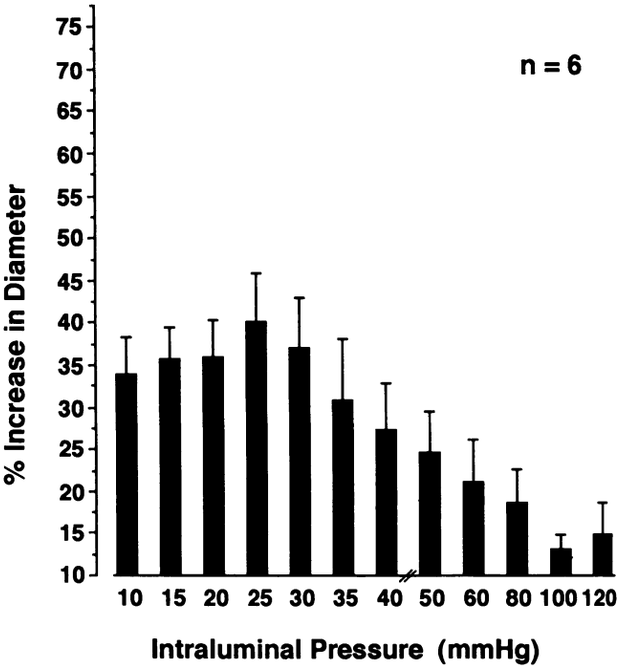

FIGURE 3. *Contractions in response to 100 mmol/L KCl in mesenteric resistance arteries obtained from normotensive humans. Maximal contraction occurred at a perfusion pressure of 25 mm Hg. In the higher pressure range, the response decreased significantly (P < .01 with a pressure of > 35 v 25 mm Hg).*

Vascular Dimensions Under baseline conditions (10 mm Hg), the internal diameter of human mesenteric resistance arteries averaged 214 ± 27 μm and the wall thickness 36 ± 4 μm, (Figure 1, n = 7). The WKY arteries had a comparable inner diameter of 201 ± 6 μm and a wall thickness of 32 ± 4 μm (n = 7). The SHR vessels of the same type showed a slightly smaller lumen diameter (172 ± 6 μm) and exhibited a significant increase in wall thickness (47 ± 2 μm; n = 6; $P <$ .001, Figure 1). Hence, the internal radius/wall thickness ratio was comparable in human and WKY arteries (3.39 ± 0.88 and 3.39 ± 0.41), but significantly smaller in the SHR (1.95 ± 0.11; $P <$ .01).

Effects of Intraluminal Pressure on Internal Vascular Diameter With perfusion pressure increasing from 10 to 120 mm Hg, unstimulated vessel diameter increased in all three groups studied (Figure 2). Human resistance arteries showed a steep increase of internal diameter in response to the increase of pressure from 10 to 25 mm Hg. Above 25 mm Hg, inner diameter remained almost constant. In contrast, the WKY arteries had a flatter and more linear pressure-diameter relationship over the whole pressure range. In SHR vessels, tbe pressure-distension relationship was significantly flatter than that in WKY ($P <$ .001, Figure 2).

Contractile Properties At a perfusion pressure of 10 mm Hg, KCl (100 mmol/L) evoked a contraction of 34 ± 3% of the baseline diameter of human resistance arteries (n = 7). With increasing intraluminal pressure, the contractile response of the human arteries increased up to a maximum of 40 ± 5% at 25 mm Hg. Above 25 mm Hg, the contractions declined to a minimum of 13 ± 7% at 100 mm Hg ($P <$ .01 in the pressure range over 35 mm Hg, compared with 25 mm Hg, Figure 3).

Compared with human arteries, vessels obtained from WKY showed an augmented contractility in the entire pressure range (63 ± 3% at 10 mm Hg; n = 6; $P <$ .01, Figure 4). The contractile response increased with increasing intraluminal pressure and reached a maximum at 25 mm Hg (72 ± 4%). As in human arteries, a high-pressure range was associated with a decrease in contractility to a minimum at 120 mm Hg (45 ± 5%). In contrast to human arteries, in which we noted the decrease in the pressure range above 35 mm Hg, in WKY this decrease only reached statistical significance in the pressure range over 80 mm Hg ($P <$ .01) compared with 25 mm Hg (Figure 4).

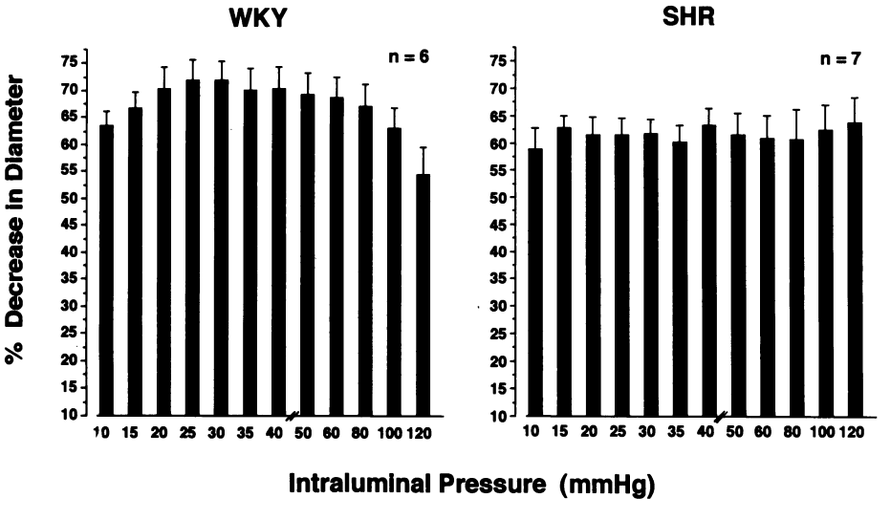

FIGURE 4. *Contractions in response to 100 mmol/L KCl in mesenteric resistance arteries obtained from WKY and SHR. In WKY, the contractions showed a pressure-dependent increase and decrease ($P < .01$ at pressures < 15 and > 80 mm Hg v 25 mm Hg). In contrast to the WKY, the contractions in SHR resistance arteries were similar over the entire pressure range studied, but tended to be maximal at 120 mm Hg.*

In contrast to the resistance arteries of normotensive humans and WKY rats, the contractions of SHR vessels were not influenced by intraluminal pressure, at least up to a pressure of 120 mm Hg. On the contrary, contractions in SHR tended to be higher at a perfusion pressure of 120 mm Hg (64 ± 4% at 120 mm Hg *v* 59 ± 4% at 10 mm Hg; P = NS; Figure 4).

DISCUSSION

This study demonstrates that perfusion pressure profoundly affects passive and active vascular properties of perfused isolated mesenteric resistance arteries of humans and normotensive rats. In hypertensive mesenteric resistance arteries, the pressure-dependent modulation of vascular activity is lost.

In human arteries, increasing intraluminal pressure was associated with a nonlinear increase in intraluminal diameter. Although the diameter increased steeply in the lower pressure range (up to 25 mm Hg), vascular dimensions remained almost constant in the upper pressure range. Although the vascular dimensions (ie, lumen/wall ratio) were comparable in human and WKY mesenteric resistance arteries, the pressure-diameter relationship differed. In particular, in the low pressure range, compliance of human arteries tended to be greater than in WKY, whereas in the upper pressure range—where the diameter of human arteries remained almost constant—rat resistance arteries still exhibited an approximately linear distension behavior. The different passive behaviors of human and rat arteries to increasing intraluminal pressure in the lower range are most likely related to different elastic properties of the vessel wall, although a small active component cannot be excluded. The composition of matrix substances in the media or adventitia may differ among the two species.[10] However, the difference in the upper pressure range with an almost constant diameter despite increasing intraluminal pressure in human arteries, but a near-linear distension behavior of rat arteries, must be related to different contractile properties in response to increasing intraluminal pressure. These functional differences may be species-specific or related to different anatomic locations of rat and human arteries. Indeed, mesenteric arteries with a diameter of 200 μm in humans may represent blood vessels of the fourth or fifth order in the mesenteric vascular bed, whereas arteries of the same size obtained form the rat were of the third order and thus may still have some conduit function. This could explain why human arteries exhibited a different distension behavior in the higher pressure range.

Compared with WKY, the SHR arteries of the same branch had a reduced internal diameter and an increased wall thickness. This increased of wall thickness has been shown to result mainly from medial hypertrophy, increased cell volume and/or remodeling.[11] This may explain the flatter pressure-diameter relationship in SHR compared with WKY as also observed by others.[12] Indeed, according to the Laplace law, medial hypertrophy is associated with reduced wall tension at any given pressure level, particularly with a decreased internal diameter.

To assess contractile responses of mesenteric resistance arteries, we used potassium chloride in a concentration (100 mmol/L) previously shown to be maximal for this agonist in this preparation.[13]

The contractility of human and WKY arteries was markedly influenced by intraluminal pressure suggesting that—as in the heart[14,15]—stretch exerted to the smooth muscle cells of the vascular media determined their contractile response. In human mesenteric resistance arteries, the highest contractions to KCl occurred at

an intraluminal pressure of 25 mm Hg. Between 30 and 80 mm Hg, their contractile capacity decreased markedly. To our knowledge, blood pressure has never yet been measured directly in human mesenteric resistance arteries with a diameter of 200 μm. As judged from the rat, and taking into account the much longer distance between the aorta and resistance arteries of that size in man, blood pressure has been estimated to be in the range of 40 to 60 mm Hg.[16] This corresponds well to the pressure range where the contractility of human mesenteric arteries was most sensitive to small changes of intraluminal pressure. Thus, this pressure dependence of the contractile behavior of mesenteric resistance arteries may represent a regulatory mechanism of this circulatory bed to protect systemic hyper- and hypotension. A similar pressure-dependent modulation of the contractility was found in WKY. The pressure range where the most effective modulation of the contractility occurred was shifted to a higher level (ie, 60 to 120 mm Hg), which corresponds well to the physiologic blood pressure in these rat resistance arteries.

Traditionally, vascular reactivity of resistance arteries has been determined in myograph systems[17] with measurement of isometric tension rather than active changes in vascular diameter (ie, vasomotion), as in the arteriograph system used in this study. In isolated rings suspended in myograph systems under no-flow conditions, optimal isometric responses are obtained at a passive tension in the range of 100 mm Hg.[17] In the arteriograph system, it appears that the contractile responses reached their optimal levels at a much lower pressure, most likely because of the presence of flow and different vascular geometries of perfused and pressurized arteries. In addition to the pressure-dependent modulation of vascular reactivity observed in our study, physical forces, in particular flow-dependent vasodilation and myogenic responses, also contributed to the regulation of resistance artery tone.[1–4] Indeed, shear forces exerted by the circulating blood or perfusion solution activate both the endothelium and vascular smooth muscle, respectively, an effect which must be absent in rings stretched in myograph systems. This makes it difficult to compare directly results obtained in arteriographs and myographs. It is obvious that in perfused and pressurized resistance arteries, multiple mechanisms take part in the regulation of vascular diameter. Indeed, during the course of active contraction vascular diameter flow decreases and, as a consequence, shear forces must change, although pressure remains constant. On the other hand, at different pressures flow changes even without alterations in vascular diameter.

In hypertensive resistance arteries with increased wall thickness, pressure-dependent modulation of vascular contractility was lost. In fact, SHR resistance arteries exhibited maximal contractility at a perfusion pressure of 120 mm Hg. In SHR arteries of this size, blood pressure has been estimated to be in the range of 80 to 110 mm Hg.[16] The loss of pressure-dependent modulation of vascular contractility in SHR may be caused by the increased wall thickness with decreased vascular compliance and a marked shift of the active force-distension relationship to higher pressure. This would result in a lower wall stress at each pressure level, which in turn would limit the modulatory effects of intraluminal pressure.

Thus, our study underscores the importance of an increased wall thickness in hypertension and demonstrates that structural changes markedly alter the pressure-dependent modulation of vascular diameter and contractility. Especially in experiments with human resistance arteries, this influence of pressure-dependent modulation on results must be considered carefully. In addition, these results demonstrate that, in experimental studies, differences in contractility between WKY and SHR may be present or absent depending on the intraluminal pressure used. Indeed, SHR mesenteric resistance arteries may surpass the contractility of the WKY in experiments with high intraluminal pressure, whereas in the lower pressure range, the WKY arteries exhibit a greater contractility.

ACKNOWLEDGMENTS

The authors thank Mrs. Monika Hermle and Mrs. Erika Weber for their technical assistance and Mrs. Bernadette Libsig for her help in the preparation of the figures.

REFERENCES

1. Rubanyi GM, Romeros C, Vanhoutte PM: Flow-induced release of endothelium-derived relaxing factor. Am J Physiol 1986;250:H1145–H1149.
2. Tesfamariam B, Halpern W, Osol G: Luminal flow enhances reactivity of small arteries in vitro. Fed Proc 1985;44:433.
3. Tesfamariam B, Halpern W, Osol G: Effects of perfusion and endothelium on the reactivity of isolated resistance arteries. Blood Vessel 1985;22:301–305.
4. Bevan JA, Joyce EH, Wellman GC: Flow-dependent dilation in a resistance artery still occurs after endothelium removal. Circ Res 1988;63:980–985.
5. Hayashi K, Epstein M, Loutzenhiser R: Pressure-induced vasoconstriction of renal microvessels in normotensive and hypertensive rats. Circ Res 1989;65:1475–1484.
6. Folkow B: Physiological aspects of primary hypertension. Am J Physiol 1982;62:348–509.
7. Halpern W, Osol G, Coy GS: Mechanical behavior of pressurized in vitro pre-arteriolar vessels determined with a video dimensions system. Ann Biomed Eng 1984;12:463–479.
8. Dohi Y, Thiel MA, Bühler FR, Lüscher TF: Activation of

endothelial L-Arginine pathway in resistance arteries. Hypertension 1990;15:170–179.

9. Snedecor GW, Cochran WG: Statistical Methods. 7th ed. Iowa City, Iowa State University Press, 1980.
10. Scott-Burden T, Resink TJ, Bürgin M, Bühler FR: Extracellular matrix: differential influence on growth and biosynthesis patterns of vascular smooth muscle cells from SHR and WKY rats. J Cell Physiol 1989;141:267–274.
11. Mulvaney MJ, Baandrup U, Gundersen HJG: Evidence for hyperplasia in mesenteric resistance vessels of spontaneously hypertensive rats using a three-dimensional dissector. Cir Res 1985;57:794–800.
12. Biochemical and mechanical properties of resistance arteries from normotensive and hypertensive rats. Hypertension 1983;5:17–25.
13. Asano M, Aoki K, Matsuda T: Quantitative changes of maximum contractile response to norepinephrine in mesenteric arteries from spontaneously hypertensive rats during the development of hypertension. Cardiovasc Pharmacol 1984;6:727–731.
14. Frank O: Zur Dynamik des Herzmuskels. Z Biol 1885;32:370.
15. Starling EH: Linacre Lecture on the Law of the Heart. London, Logmans, Green & Co, 1918.
16. Bohlen HG, Core RW, Hutchins PH: Comparison of microvascular pressures in normal and spontaneously hypertensive rats. Microvasc Res 1977;13:125–130.
17. Mulvany MJ, Halpern W: Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 1977;41:19–26.