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Endothelial Dysfunction Augments Myogenic Arteriolar Constriction in Hypertension

An Huang, Dong Sun, Akos Koller

To elucidate the underlying reason or reasons for the increased peripheral resistance in hypertension, we investigated the pressure-diameter relation—the myogenic response—of isolated, cannulated arterioles (approximately 50 μm) of cremaster muscle of 12-week-old Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR), and normal Wistar (NW) rats. All arterioles constricted in response to step increases in perfusion pressure from 20 to 160 mm Hg. This constriction was, however, significantly enhanced from 60 to 160 mm Hg in arterioles of SHR compared with NW or WKY rats. For example, at 80 and 140 mm Hg, respectively, the normalized diameter (expressed as a percentage of the corresponding passive diameter of arterioles of SHR) was 11.8% and 27.6% ($P<.05$) less compared with those of WKY rats. Endothelium removal eliminated the enhanced pressure-induced tone in SHR. Similarly, indomethacin (10^{-5} mol/L, sufficient to block prostaglandin synthesis) or SQ 29,548 (10^{-6} mol/L), a thromboxane A_2–prostaglandin H_2 receptor blocker that inhibited vasoconstriction to the thromboxane agonist U46619, attenuated the enhanced pressure-diameter curve and reversed the blunted dilation to arachidonic acid in SHR. In contrast, the thromboxane A_2 synthesis inhibitor CGS 13,080 (5×10^{-6} mol/L) did not affect the increased pressure-induced tone or the reduced dilation to arachidonic acid in SHR. Thus, the present findings suggest that in early hypertension pressure-induced arteriolar constriction is increased. This seems to be due to an enhanced production of endothelium-derived constrictor factors, primarily prostaglandin H_2. (*Hypertension*. 1993;22:913-921.)

KEY WORDS • myogenic response • arachidonic acid • prostaglandins • acetylcholine • nitroprusside • indomethacin • receptors, thromboxane

In vivo studies of the microcirculation of skeletal muscle in hypertension have demonstrated a variety of changes in the vascular wall and network architecture, which are believed to be a consequence of the increased intravascular pressure.[1-8] These structural changes are thought to contribute to the maintenance of increased peripheral vascular resistance.[5,6] Although in previous studies[9-12] the changes in the vasoactive function of large arteries in various forms of hypertension have been amply demonstrated, such investigations are sparse in microvessels.[13,14]

One of the primary local mechanisms that regulate arteriolar diameter is the myogenic response.[15-17] Because this mechanism is activated by the transmural pressure, it is logical to assume that it is altered by the presence of an increased pressure in hypertension. Indeed, an augmented vasoconstrictor response to changes in intravascular pressure is characteristic of patients with essential hypertension.[18] Although in most microvascular beds the myogenic response is intrinsic to vascular smooth muscle,[17,19,20,21] in some vessels the endothelium seems to have an obligatory role or contributes significantly to it.[22,23] Based on these studies we hypothesized (1) that the chronic presence of high intravascular pressure in hypertension may alter pres-

sure-sensitive vascular mechanisms, resulting in an enhanced arteriolar tone and (2) that endothelial factors contribute to the enhanced pressure-induced constriction of arterioles.

To test these ideas we investigated the changes in arteriolar diameter as a function of intravascular pressure in static flow conditions in isolated cremasteric arterioles of normotensive and spontaneously hypertensive rats (SHR). In addition, the possible role of the endothelium of arterioles in the development and/or modulation of myogenic constriction was assessed by removal of the endothelium and the use of pharmacologic agents affecting the cyclooxygenase pathway.

Methods

Experiments were conducted on isolated second-order arterioles (approximately 50 μm) of cremaster muscle of 12-week-old male normal Wistar (NW) rats, Wistar-Kyoto (WKY) rats, and SHR. The procedures and protocols of study were in accordance with our institutional guidelines. Systolic arterial blood pressure was measured by the tail-cuff method. Rats were anesthetized with intraperitoneal injections of sodium pentobarbital (Nembutal sodium, 50 mg/kg). The isolation procedure of cremasteric arterioles has been described previously.[21] Briefly, the cremaster muscle of rats was exposed by a median incision of the scrotal sac, cleared of adhering fascia, and separated from the scrotal sac. The muscle then was cut out and placed on a Petri dish containing cold (0° to 4°C) salt solution (pH 7.4) composed of (mmol/L) NaCl, 145; KCl, 5.0; $CaCl_2$, 2.0;

$MgSO_4$, 1.0; NaH_2PO_4, 1.0; dextrose, 5.0; pyruvate, 2.0; EDTA, 0.02; and 3-(N-morpholino)propanesulfonic acid, 3.0. The muscle was pinned to the silicone bottom of the dish and allowed to equilibrate for approximately 15 minutes. After the muscle had been dissected, rats were euthanized by an overdose of pentobarbital sodium.

With the use of microsurgery instruments and an operating microscope (Olympus), four segments, 1 to 2 mm in length, of second-order arterioles branching off from the main (first-order) arteriole supplying the muscle were isolated sequentially from both cremaster muscles then transferred to the vessel chamber. The chamber contained four pairs of glass micropipettes filled with physiological salt solution (PSS) (see below) at room temperature.

The four proximal (inflow) micropipettes were connected to four silicone rubber tubes linked to a pressure-servo syringe system (Living Systems Inc, Burlington, Vt). All distal (outflow) pipettes were equipped with a three-way stopcock. PSS used for suffusion and perfusion of the vessels contained (mmol/L) NaCl, 110.0; KCl, 5.0; $CaCl_2$, 2.5; $MgSO_4$, 1.0; dextrose, 10.0; $NaHCO_3$, 24.0; and EDTA, 0.02 and was equilibrated with a gas mixture of 21% O_2 and 5% CO_2 balanced with N_2, at pH 7.4 (33°C). From a reservoir, the vessel chamber (20 mL) was continuously supplied with PSS at a rate of 40 mL/min.

After all four vessels had been mounted on their proximal pipettes and secured with sutures, perfusion pressure was raised to 20 mm Hg to clear clotted blood from the lumens, then the other end of the vessels was mounted on the distal pipettes. To flush the vessels and cannulas, the system was perfused for several minutes, then the outflow cannulas were closed, and the perfusion pressure was slowly (approximately 1 minute) increased to 80 mm Hg. At this time, the pressure-servo system was placed in the manual mode (ie, no automatic maintenance of perfusion pressure) to ascertain that there were no leaks in the system. If no leaks were detected (ie, perfusion pressure remained constant), the pressure-servo system was set in the automatic mode. The temperature was set to 33°C (YSI temperature controller), and the vessels were allowed to equilibrate for approximately 1 hour.

Experimental Procedure

In all protocols, the changes in diameter of arterioles in response to increases in perfusion pressure under no-flow conditions were measured with a reticle generator and recorded with an X-Y recorder. Only those vessels that developed spontaneous tone to pressure (80 mm Hg) were used, as there was no vasoactive agent added to the PSS. After the equilibration period, perfusion pressure was decreased to 20 mm Hg and then increased in steps of 20 mm Hg to 160 mm Hg. Each pressure step was maintained for 5 to 10 minutes to allow the vessels to reach a stable condition before the diameter of the arterioles was measured. After the pressure-diameter relation was obtained, the pressure was lowered to 80 mm Hg, then after approximately 20 minutes responses of arterioles to vasoactive agents were tested.

In the first protocol after control responses, the endothelium of the arterioles was removed by perfusion

of the vessels with air, as previously described in detail.[21] The arteriole was untied from the proximal pipette, and air (approximately 1 mL) was injected through the distal pipette into the lumen for 1 minute. Then the arteriole was remounted to the proximal pipette. This procedure was repeated in each vessel. The arterioles were then perfused with PSS for 20 minutes at a pressure of 20 mm Hg to clear the debris. The outflow stopcock was then closed and perfusion pressure raised to 80 mm Hg for 30 minutes for retrieval of a stable tone. At this pressure the efficacy of endothelial denudation was ascertained by testing the arteriolar responses to acetylcholine (5×10^{-8} mol/L) and sodium nitroprusside (10^{-7} mol/L), endothelium-dependent and -independent vasodilators,[24,25] respectively, before and after the administration of the air bolus. After removal of the endothelium, changes in diameter in response to step increases in perfusion pressure were reassessed.

In the second series of experiments, after control pressure-diameter curves were obtained, the vessels were subjected to indomethacin (10^{-5} mol/L), an inhibitor of prostaglandin synthesis, instead of endothelial denudation. Then changes in diameter in response to step increases in perfusion pressure were reassessed. Arteriolar responses to arachidonic acid (10^{-5} mol/L) and prostaglandin E_2 (PGE_2, 5×10^{-8} mol/L) were obtained before and after the vessels were exposed to indomethacin.

In the third group of experiments, the pressure-diameter relation was investigated in control conditions and in the presence of SQ 29,548 (10^{-6} mol/L), known to be a thromboxane A_2 (TXA_2)–prostaglandin H_2 (PGH_2) receptor antagonist.[26] The efficacy of this antagonist was tested by U46619 (10^{-8} mol/L), a TXA_2-PGH_2 receptor agonist. In the fourth group of experiments the effect of CGS 13,080 (5×10^{-6} mol/L), an inhibitor of TXA_2 synthetase,[27] on the pressure-induced changes in diameter was assessed. In both the third and fourth groups of experiments, dilation in response to arachidonic acid was also tested.

All drugs were added to the reservoir connected to the vessel chamber, and final concentrations are given. Responses to vasoactive agents were tested at 80 mm Hg perfusion pressure. At the conclusion of each experiment, the suffusion solution was changed to a Ca^{2+}-free PSS that contained sodium nitroprusside (10^{-4} mol/L) and EGTA (1.0 mmol/L). The vessels were incubated for 10 minutes, then the step increases in pressure were repeated, and the passive diameter of arterioles at each pressure step was obtained.

All salts and chemicals were obtained from Sigma Chemical Co, St Louis, Mo, or Aldrich Chemical Co, Milwaukee, Wis, and were prepared on the day of the experiment. Changes in diameter in response to vasoactive agents or pressure were normalized to the corresponding passive diameter and expressed as percent changes. Results are presented as mean±SEM. N and n refer to the number of rats and vessels, respectively. Statistical analyses were done by analysis of variance (random and fixed models were used for data of myogenic responses within groups and between groups, respectively), followed by Tukey's post hoc test, regression analysis, and Student's t test (paired and grouped t tests) were used for data of drug responses within and

between groups, respectively), as appropriate. A value of $P<.05$ was considered significant.

Results

The systolic blood pressures of NW and WKY rats and SHR were 103.9±3.0 (n=6), 119.1±7.8 (n=6), and 194.9±3.0 (n=7) mm Hg, respectively, showing a significant increase in blood pressure in SHR compared with NW and WKY rats. There was no significant difference between the systolic pressure of NW and WKY rats.

In the first series of experiments, the diameter of NW, WKY, and SHR arterioles as a function of perfusion pressure was obtained in the presence and absence of endothelium. To assess the magnitude of the pressure-induced changes in diameter, we also obtained after the conclusion of experiments the passive pressure-diameter relation of arterioles in Ca^{2+}-free solution in all three rat strains (see "Methods").

Fig 1 (top) demonstrates the changes in the normalized diameter of NW, WKY, and SHR arterioles in response to step increases in perfusion pressure. The changes in diameter at each pressure step were normalized to the corresponding passive diameter. From 40 mm Hg perfusion pressure, the slopes of the pressure-diameter curve of SHR arterioles started to deviate significantly ($P<.05$) from those of NW and WKY arterioles (Fig 1, top panel inset), indicating that SHR arterioles have an enhanced constriction in response to step increases in perfusion pressure compared with arterioles of normotensive rats. Also, in the range of 80 to 160 mm Hg pressure, the diameter of SHR arterioles was significantly less than those of normotensive rats. This finding was further analyzed in the middle panel of Fig 1. We found that the differences between the normalized diameter of normotensive and hypertensive rats increased as a function of intravascular pressure, as indicated by the significant correlation of these two parameters. The bottom panel of Fig 1 shows that although the diameters of arterioles of the various rat strains were the same at 20 mm Hg pressure, the pressure–passive diameter relations of SHR and WKY arterioles were significantly different from that of NW arterioles.

To assess the role of endothelium in the enhanced constriction to pressure, we removed the endothelium of arterioles. As in previous studies[20,21] we found that infusion of air resulted in loss of the endothelium, as indicated by the absence of dilation to acetylcholine while dilation to sodium nitroprusside remained intact (Table 1). Fig 2 (middle) shows that after endothelium removal, the pressure-diameter curve of WKY rats shifted downward; that is, the constriction of the arterioles in response to pressure was significantly enhanced. In contrast, in SHR (Fig 2, bottom), after removal of the endothelium the pressure-diameter curve of arterioles shifted significantly upward; that is, the constriction of vessels to pressure was significantly reduced.

Next we investigated the endothelial mechanism or mechanisms responsible for the pressure-induced enhanced constriction of SHR arterioles. Dilator responses of arterioles to arachidonic acid were significantly blunted in SHR compared with NW or WKY rats, and dilation to PGE$_2$ was also reduced in both WKY rats and SHR compared with NW rats (Table 2).
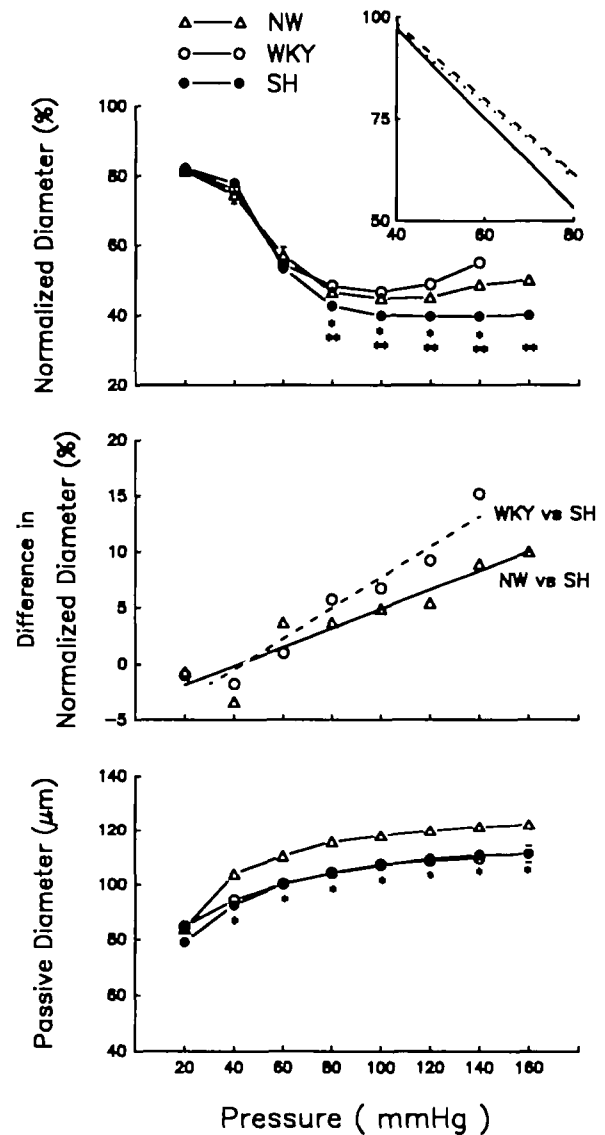

FIG 1. Top: Line graph shows comparison of pressure-induced changes in normalized diameter of endothelium-intact (control) arterioles of normal Wistar (NW) (N=25 rats, n=44 vessels), Wistar-Kyoto (WKY) (N=24, n=46), and spontaneously hypertensive (SH) (N=28, n=55) rats. Changes in diameter were normalized to passive diameter at the corresponding perfusion pressures. Analysis of variance indicates that the slope of pressure-diameter curves of arterioles was significantly different ($P<.05$) between 40 and 80 mm Hg pressure, as also illustrated by the linear regression lines in the inset. Inset: Diameters were normalized to 40 mm Hg pressure. Dashed, dotted, and solid lines represent data of NW, WKY, and SH rats, respectively. NW: $y=-0.91x+134.8$, $r=.99$; WKY: $y=-0.89x+132.2$, $r=.95$; SH: $y=-1.10x+141.0$, $r=.98$. Middle: Line graph shows differences in normalized arteriolar diameter as a function of perfusion pressure in NW and SH and WKY and SH rats, respectively. Regression lines $y=0.085x-35.8$ and $y=0.13x-5.67$ have significant r values of .93 and .96, respectively ($P<.01$). Bottom: Line graph shows comparison of pressure–passive diameter curves of arterioles of various rat strains. *Significant difference between NW and WKY or SHR rats. Data are mean±SEM.

TABLE 1.   Effect of Endothelium Removal on Arteriolar
Responses to Acetylcholine and Sodium Nitroprusside

Rat Strain	Acetylcholine		Sodium Nitroprusside	
	Control	−E	Control	−E
NW (N=5, n=9)	33.3±3.6	−1.3±0.4*	22.4±2.9	22.7±2.7
WKY (N=6, n=8)	30.3±4.2	−1.0±0.7*	17.2±1.1†	16.2±1.0†
SHR (N=6, n=10)	30.3±2.54	−0.8±0.3*	15.6±1.3†	16.2±1.4†

−E indicates endothelium removed; NW, normal Wistar rats; WKY, Wistar-Kyoto rats; and SHR, spontaneously hypertensive rats. Data are mean±SEM of percent changes in arteriolar diameter in response to 5×10^{-8} mol/L acetylcholine and 10^{-7} mol/L sodium nitroprusside before (control) and after removal of endothelium. N is number of rats; n is number of vessels.

*Significant difference ($P<.05$) from control.

†Significant difference ($P<.05$) of arteriolar responses of WKY rats and SHR from those of NW rats in the same conditions.

The efficacy of the inhibition of cyclooxygenase by indomethacin is indicated by the elimination of the dilation to arachidonic acid in all three rat strains. Indomethacin restored the dilation to PGE_2 in SHR comparable to the responses in NW arterioles (Table 2). In normotensive rats indomethacin slightly enhanced pressure-induced arteriolar tone, but except at 160 mm Hg pressure in NW arterioles, there were no significant differences between the pressure-diameter curves of NW and WKY arterioles before or after inhibition of cyclooxygenase (Fig 3, top and middle). In contrast, in SHR arterioles, indomethacin significantly reduced the arteriolar constriction in response to step increases in perfusion pressure (Fig 3, bottom).

In other experiments the effect of SQ 29,548, a TXA_2-PGH_2 receptor blocker, on the pressure-induced behavior of arterioles was examined. SQ 29,548 eliminated the arteriolar constriction to U46619, known to be a specific agonist of this receptor in all three rat strains, and normalized the diminished dilation to arachidonic acid in SHR arterioles (Table 3). Also, although SQ 29,548 did not affect pressure-diameter relations of NW and WKY arterioles, it significantly reduced the pressure-induced arteriolar constriction of SHR (Fig 4).

To further elucidate the nature of the endothelial mediator involved in the enhanced arteriolar constriction in SHR, we investigated the effects of CGS 13,080, a TXA_2 synthesis blocker, on the pressure-diameter curve of arterioles of various rat strains. Table 4 shows that this compound affected neither the constriction to U46619 nor the blunted dilation to arachidonic acid in SHR. We also found that although CGS 13,080 elicited a reduction in arteriolar diameter at low intravascular pressures in all rat strains (most likely a nonspecific effect), it did not affect the pressure-diameter curve of arterioles (Fig 5).

In all groups of experiments we found that dilation to arachidonic acid was blunted somewhat in WKY compared with NW arterioles, but this difference reached significance only when all data were combined. In this case, dilation to arachidonic acid of NW arterioles was significantly greater (28.9±1.9%; N=20, n=30) than
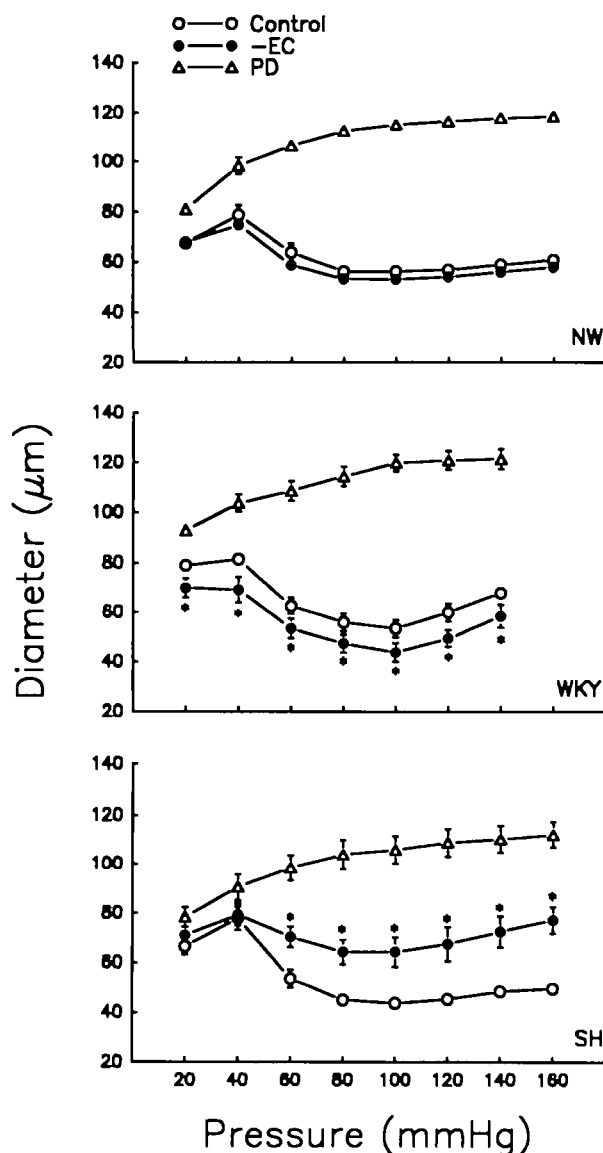

FIG 2.   Line graphs show arteriolar diameter as a function of perfusion pressure of normal Wistar (NW) (N=5 rats, n=9 vessels), Wistar-Kyoto (WKY) (N=6, n=10), and spontaneously hypertensive (SH) (N=6, n=10) rats before and after removal of endothelium. Open circles represent diameter of arterioles with endothelium (control); filled circles represent diameter of arterioles without endothelium (−EC); open triangles represent passive diameter (PD) of arterioles in Ca^{2+}-free solution (see "Methods"). Data are mean±SEM. *Statistically significant differences ($P<.05$) between arteriolar diameters with (control) and without (−EC) endothelium at corresponding perfusion pressures.

that of WKY arterioles (22.5±1.5%; N=18, n=36) ($P<.05$).

Discussion

The salient findings of this study are that in young SHR the diameter of arterioles at each pressure step is significantly less than that of normotensive rats. The enhanced constriction of arterioles in hypertensive rats correlates significantly with increases in perfusion pres-

TABLE 2. Effect of Indomethacin on Arteriolar Responses to Arachidonic Acid and Prostaglandin E_2

Rat Strain	Arachidonic Acid		Prostaglandin E_2	
	Control	Indo	Control	Indo
NW (N=7, n=11)	30.5±2.4	0.8±0.3*	27.2±2.1	30.8±0.3
WKY (N=6, n=16)	25.4±2.5	0.1±0.3*	19.2±1.6†	21.1±1.4†
SHR (N=8, n=21)	13.3±3.2†‡	0.4±0.3*	18.8±1.8†	25.7±2.0*†

Indo indicates indomethacin; NW, normal Wistar rats; WKY, Wistar-Kyoto rats; and SHR, spontaneously hypertensive rats. Data are mean±SEM of percent changes in arteriolar diameter in response to 10^{-5} mol/L arachidonic acid and 5×10^{-6} mol/L prostaglandin E_2 in control and after treatment with 10^{-5} mol/L indomethacin. N is number of rats; n is number of vessels.

*Significant difference ($P<.05$) from control.

†Significant difference ($P<.05$) from responses of NW rats in the same conditions.

‡Significant difference ($P<.05$) between WKY rats and SHR in the same conditions.

sure. Moreover, the enhanced constriction of arterioles is primarily due to the continuous release of an endothelium-derived constrictor prostanoid.

Previous investigation of the microcirculation of hypertensive animals revealed structural changes in the vascular wall as well as architectural changes in the arteriolar network.[1-8,28] These changes were used to explain the enhanced peripheral resistance that accompanies hypertension.[5,6] The question our study addressed was whether alterations in the function of arteriolar endothelium and/or smooth muscle in the development of pressure-induced tone could be observed in isolated arterioles of young genetically hypertensive rats and if so, whether this could be related to the elevated intravascular pressure. Arterioles of rat cremaster muscle were chosen for the present study because many previous studies investigating the effects of hypertension on the microcirculation have been conducted in this vascular bed.[1-4] Also, in this preparation micropressure measurements in vivo showed that intravascular pressure was elevated in second-order arterioles (approximately 50 μm) of SHR compared with normotensive rats,[29,30] the same vessels we used in the present study.

To avoid the interference of other local mechanisms, besides the pressure-sensitive mechanism, that regulate arteriolar diameter, we investigated the presence of and changes in the myogenic mechanism in isolated cannulated arterioles under no-flow conditions. We used relatively young 12-week-old animals, an age at which SHR already have a significantly elevated blood pressure compared with NW or WKY rats.

In response to increases in perfusion pressure, arterioles of hypertensive rats exhibited an enhanced constriction compared with normotensive rats. The increase in tone was eliminated by removal of the endothelium (Fig 2, bottom), indicating that arteriolar endothelium of hypertensive rats produces a factor or factors that constrict arteriolar smooth muscle and are responsible for the enhanced constriction. Furthermore, the positive correlation between the increase in pressure and the additional constriction (Fig 1, middle) suggests that
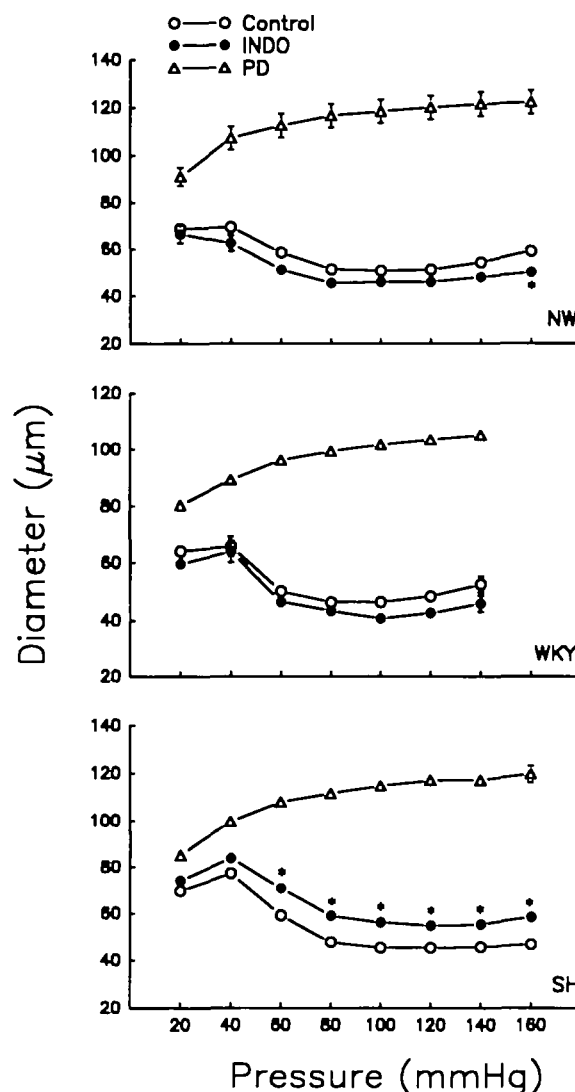

FIG 3. Line graphs show effect of indomethacin (INDO, 10^{-5} mol/L) on pressure-diameter relations in arterioles of normal Wistar (NW) (N=7 rats, n=12 vessels), Wistar-Kyoto (WKY) (N=6, n=16), and spontaneously hypertensive (SH) (N=8, n=21) rats. Data are mean±SEM. *Statistically significant differences ($P<.05$) between arteriolar diameters in control condition and presence of indomethacin. PD, passive diameter.

the synthesis of the endothelial factor or factors is coupled to the increases in pressure. Release of a constrictor factor from the endothelium in response to pressure[23] was reported previously in cerebral arteries of normotensive rats but as yet not in other vascular beds.

Removal of the endothelium moderated the pressure-induced constriction of WKY but not NW arterioles, indicating a continuous synthesis and release of an endothelium-derived dilator factor or factors that attenuate the myogenic tone of WKY arterioles. Our previous findings also demonstrated a modulatory role of endothelium in the myogenic tone of gracilis muscle arterioles but not of mesenteric arterioles in normotensive rats.[17,20] In the present experiments in arterioles with intact endothelium, the pressure-induced constric-

TABLE 3. Effects of SQ 29,548 on Arteriolar Responses to Vasoactive Agents

Rat Strain	U46619		Arachidonic Acid	
	Control	SQ 29,548	Control	SQ 29,548
NW (N=8, n=11)	−13.4±1.2	−0.9±0.2*	26.7±2.4	29.2±4.5
WKY (N=7, n=12)	−13.8±0.8	−1.4±0.4*	20.7±2.2	25.2±2.3
SHR (N=8, n=13)	−14.8±0.6	−0.5±0.2*	9.0±1.4†‡	30.1±2.6*

NW indicates normal Wistar rats; WKY, Wistar-Kyoto rats; and SHR, spontaneously hypertensive rats. Data are mean±SEM of percent changes in diameter of arterioles in response to 10^{-8} mol/L U46619 and 10^{-5} mol/L arachidonic acid before (control) and after 10^{-6} mol/L SQ 29,548. N is number of rats; n is number of vessels.
*Significant difference ($P<.05$) in responses between control and in the presence of SQ 29,548.
†Significant difference ($P<.05$) between NW rats and SHR in the same conditions.
‡Significant difference ($P<.05$) between WKY rats and SHR in the same conditions.
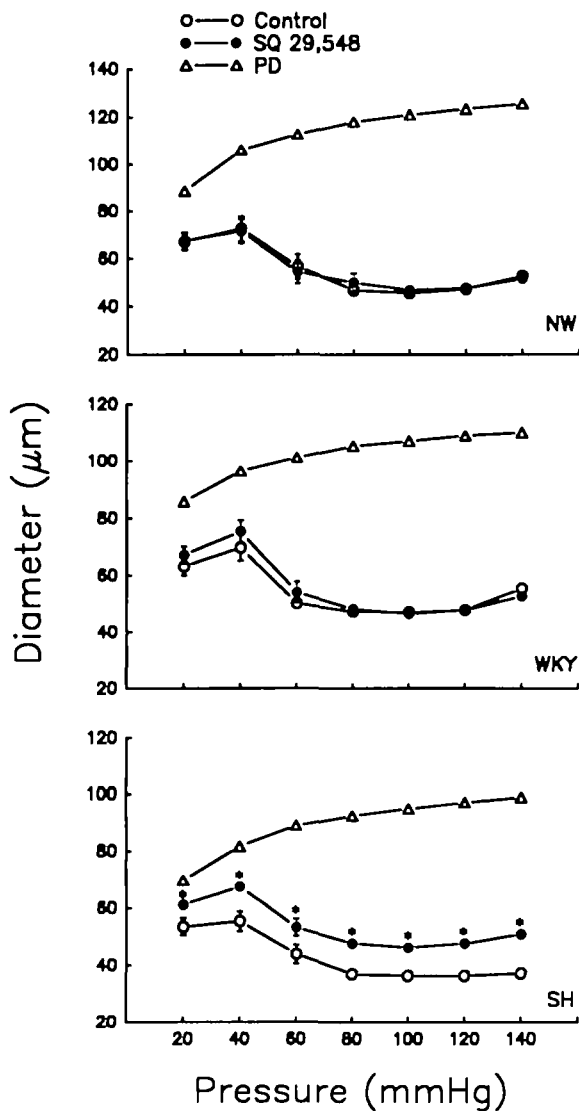

FIG 4. Line graphs show effect of SQ 29,548 (10^{-6} mol/L) on pressure-diameter relations in arterioles of normal Wistar (NW) (N=8 rats, n=14 vessels), Wistar-Kyoto (WKY) (N=7, n=12), and spontaneously hypertensive (SH) (N=8, n=13) rats. Data are mean±SEM. *Significant differences in arteriolar diameters in control and presence of SQ 29,548. PD, passive diameter.

tion of hypertensive arterioles was significantly enhanced compared with that of normotensive vessels, as indicated by the significantly steeper slopes of the pressure-diameter curves of SHR arterioles between 40 and 80 mm Hg perfusion pressure compared with those of NW and WKY arterioles (Fig 1).

All these findings indicate that endothelium has an important modulatory role in the generation of the spontaneous tone of hypertensive arterioles and that in the operational pressure range for vessels of this size[29] the responsiveness of arterioles to pressure is enhanced.

Previous studies demonstrated alterations in the function of arterial endothelium of prehypertensive SHR.[31] Because in the present experiments the blood pressure of SHR was already elevated, one cannot conclude with any certainty whether the observed change in the function of endothelium has an etiologic role in or is the result of the elevation of blood pressure. It is likely, however, that the early changes in the vasoactive function of arteriolar endothelium precede the structural changes of the arteriolar wall in this form of hypertension. Based on the measurement of passive diameters, the distensibilites of WKY and SHR arterioles do not differ significantly, but they both differ from NW arterioles (Fig 1, bottom). These findings correspond to in vivo data obtained in cremaster[1,2,29,30] and spinotrapezius[32] muscle of WKY rats and SHR, tissues in which no significant differences in average basal diameter of arterioles were found. Our findings also suggest that the changes in distensibility of SHR arterioles are not necessarily due to pressure but are perhaps genetically determined.

In previous in vivo studies, measurement of blood pressure in arterioles of skeletal (cremaster) muscle revealed that, although in the larger arterioles (approximately 80 to 150 μm) of SHR the intravascular pressures were substantially elevated compared with those of WKY rats, this difference was diminished by approaching the entry region of capillaries.[29,30] These and our studies are consistent with the suggestion that in hypertension the arterioles located in the proximal part of the microcirculation are the ones that exhibit an enhanced constriction, maintaining normal arteriolar pressure in distal parts of the network. This would help to preserve the function and structure of smaller arterioles[8] and by regulating capillary hydrostatic pressure would also assist in the maintenance of fluid balance in the capillary and postcapillary regions.

TABLE 4. Effects of CGS 13,080 on Arteriolar Responses to Vasoactive Agents

Rat Strain	U46619		Arachidonic Acid	
	Control	CGS 13,080	Control	CGS 13,080
NW (N=5, n=9)	−17.4±1.3	−13.3±0.7	29.4±4.9	25.5±3.0
WKY (N=5, n=8)	−16.2±1.3	−15.9±1.1	19.3±3.2	17.8±3.8
SHR (N=6, n=11)	−14.1±0.6	−10.8±0.7	9.3±1.5*†	7.9±1.8*†

NW indicates normal Wistar rats; WKY, Wistar-Kyoto rats; and SHR, spontaneously hypertensive rats. Data are mean±SEM of percent changes in diameter in response to 10^{-8} mol/L U46619 and 10^{-5} mol/L arachidonic acid before (control) and after administration of 5×10^{-6} mol/L CGS 13,080. N is number of rats; n is number of vessels.
*Significant difference ($P<.05$) between NW rats and SHR.
†Significant difference ($P<.05$) between NW rats and SHR in the same conditions.

Studies of the responses to vasoactive agents of ring preparations of rat aorta[10] and mesenteric arteries[11,31] demonstrated that endothelial function is altered in

FIG 5. Line graphs show effect of CGS 13,080 (5×10^{-6} mol/L) on pressure-diameter relations of arterioles of normal Wistar (NW) (N=5 rats, n=9 vessels), Wistar-Kyoto (WKY) (N=5, n=8), and spontaneously hypertensive (SH) (N=6, n=11) rats. Data are mean±SEM. *Significant differences ($P<.05$) in arteriolar diameters in control and presence of CGS 13,080. PD, passive diameter.

hypertensive animals. In these vessels responses to an endothelium-dependent dilator agent, acetylcholine, were reduced or reversed to constriction, whereas those to endothelium-independent dilator agents were retained. It was also shown that this change in the function of endothelium in hypertension might be linked to changes in arachidonic acid metabolism[33] or production of superoxide anions.[31] Although these studies revealed changes in vascular function in hypertension, the vessels were not cannulated and pressurized; rather, they were preconstricted with vasoactive agents, and therefore, the role of the observed vascular changes in pressure-induced tone development could not be elucidated. Also, because the contribution of conduit vessels to peripheral resistance is negligible, the relevance of the above findings with regard to the pathogenesis of hypertension is equivocal.

Previous studies in Dahl hypertensive rats also found a decrease in the basal release of endothelium-derived relaxing factor[13] but no appreciable change in the dilation of arterioles of spinotrapezius muscle to acetylcholine. In the present study we also did not find a diminished response to acetylcholine (Table 1), indicating that nitric oxide synthesis is preserved in the cremasteric arterioles of young SHR. Interestingly, however, dilations to sodium nitroprusside were reduced in SHR, a finding for which at present we have no explanation.

Currently, little is known concerning the changes in the myogenic response or the function of the endothelium of arterioles of skeletal muscle in hypertension. Interestingly, whereas in renal arteries of SHR myogenic tone was enhanced,[34] in Dahl (salt-sensitive) hypertensive rats renal microvessels showed impaired myogenic responses,[35] indicating that different mechanisms are present in different forms of hypertension.

That the endothelium augments the pressure-induced behavior of skeletal muscle arterioles has not been demonstrated previously. It is also important to point out that our findings were obtained during no-flow conditions, as it was shown previously that in the presence of (blood) flow the release of endothelial factors affects arteriolar tone.[21,36,37] In SHR, inhibition of prostaglandin synthesis at the level of cyclooxygenase had a significant moderating effect on the pressure-diameter relation of arterioles similar to that of removal of the endothelium, suggesting the synthesis by endothelium of a constrictor factor that is prostanoid in nature.
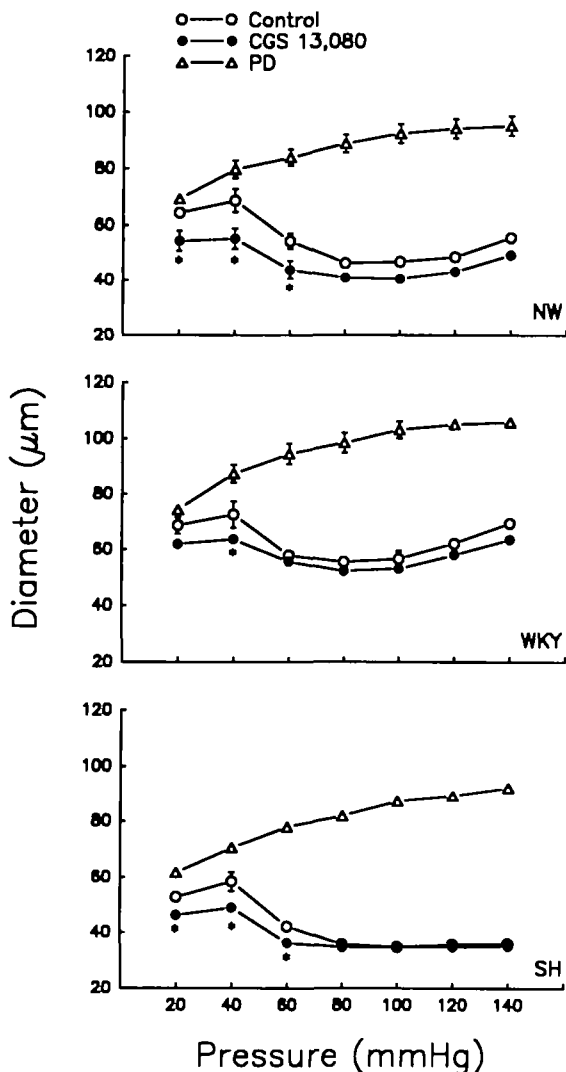
Previous studies showed that in response to the administration of arachidonic acid the endothelium of cremasteric microvessels of normotensive rats produces primarily prostaglandin I_2 (PGI_2) and PGE_2.[38] It is likely that in SHR the reduction in dilation to arachidonic acid is due to an enhanced production of a constrictor prostanoid associated with and perhaps the result of a diminished synthesis of dilator prostaglandins, such as PGI_2 and PGE_2. This idea is also supported by previous studies that showed reduced hypotensive responses to arachidonic acid in SHR.[39] In addition, the sensitivity of vascular smooth muscle to prostaglandins seems to be decreased in both SHR and WKY rats. It is noteworthy that the responses of WKY arterioles to arachidonic acid and PGE_2 but not to pressure were blunted compared with NW arterioles. Also, it seems that in WKY rats there may be an enhanced synthesis of endothelium-derived dilator factors that counteracts the increase in arteriolar tone to pressure (Fig 2, middle). In addition, WKY arterioles, unlike NW and SHR arterioles, were not able to maintain a constriction at higher intravascular pressures (Figs 1 and 2). All of these findings led us to speculate that there may be some alterations (perhaps genetic) in the structure and function in WKY arterioles that are quite like those in SHR arterioles, but because they are not exposed to high intravascular pressures in vivo, their pressure-induced responses are more like those of NW arterioles.

With the use of a TXA_2-PGH_2 receptor antagonist and a thromboxane synthesis blocker, our study revealed an alteration in the synthesis of prostaglandins in the endothelium of skeletal muscle arterioles of SHR. These experiments demonstrated that the TXA_2-PGH_2 receptor antagonist SQ 29,548 restored the normal pressure-diameter curve and the dilation to arachidonic acid. Interestingly, blockade of TXA_2 synthesis elicited a constriction at lower pressures in all rat strains, most likely because of a nonspecific effect of the inhibitor used. Nevertheless, CGS 13,080 failed to reverse the enhancement of the pressure-diameter curve and also failed to normalize dilation to arachidonic acid, suggesting that an overproduction of PGH_2 rather than TXA_2 is responsible for the enhanced myogenic tone of SHR arterioles. This conclusion is supported by previous studies which showed that activation of TXA_2-PGH_2 receptors is important in the altered responses of conduit arteries[40-42] and pial arterioles[43] of SHR to endothelium-dependent dilator agents, indicating that in hypertension changes in arachidonic acid metabolism are not unique to the skeletal muscle microcirculation.

That the constriction to a TXA_2 receptor agonist (U46619) was similar in arterioles of all three rat strains indicates no increase in the sensitivity or number of these receptors in SHR. The present findings support the hypothesis that in arterioles of genetically hypertensive rats endothelial metabolism of arachidonic acid is shifted from dilator to constrictor prostanoids, ie, that there is an enhanced synthesis of PGH_2 at the expense of PGE_2/PGI_2. Thus, one may suspect a causal relation between elevated intravascular pressure and the altered prostaglandin synthesis in arteriolar endothelium. In addition, because in SHR removal of the endothelium resulted in a greater attenuation of the myogenic tone than administration of indomethacin or SQ 29,548

(bottom panels of Fig 2 versus Figs 3 or 4), we surmise that a nonprostanoid factor, synthesized in arteriolar endothelial cells, also contributes to the enhanced pressure-induced constriction of these arterioles.

It is likely that changes similar to those described here occur in arterioles of other types of skeletal muscle. This could then lead to significant increases in peripheral resistance and blood pressure and at the same time would place greater demand on shear stress–dependent[21,37] and metabolic[44] mechanisms known to have the ability to oppose the development of a positive feedback cycle of increased myogenic constriction.

In conclusion, the present study is the first demonstration of an endothelium-dependent, enhanced pressure-induced constriction of arterioles of genetically hypertensive rats. This augmented constriction is in part due to an enhanced synthesis of PGH_2. The present findings suggest an important role for the altered vasoactive function of endothelium of skeletal muscle arterioles in the pathogenesis of hypertension.

Acknowledgments

References

1. Hutchins PM, Darnell AE. Observation of a decreased number of small arterioles in spontaneously hypertensive rats. *Circ Res*. 1974; 34,35(suppl I):I-161-I-165.
2. Chen IIH, Prewitt RL, Dowell RF. Microvascular rarefaction in spontaneously hypertensive rat cremaster muscle. *Am J Physiol*. 1981;241:H306-H310.
3. Prewitt RL, Chen IIH, Dowell RF. Development of microvascular rarefaction in the spontaneously hypertensive rat. *Am J Physiol*. 1982;243:H243-H251.
4. Hutchins PM, Dusseau JW, Marc MC, Greene PW. The role of arteriolar structural changes in hypertension. In: Henrich H, ed. *Microvascular Aspects of Spontaneous Hypertension*. Bern, Switzerland: H Huber Publishers; 1982:41-53.
5. Folkow B. Structural factor in primary and secondary hypertension. *Hypertension*. 1990;16:89-101.
6. Mulvany MJ, Aalkjaer C. Structure and function of small arteries. *Physiol Rev*. 1990;70:921-962.
7. Mulvany MJ, Hansen PK, Aalkjaer C. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media, and an increased number of smooth muscle cell layers. *Circ Res*. 1978;43:854-864.
8. Bund SJ, West KP, Heagerty AM. Effects of protection from pressure on resistance artery morphology and reactivity in spontaneously hypertensive and Wistar-Kyoto rats. *Circ Res*. 1991;68: 1230-1240.
9. Lockette WG, Otsuha Y, Carretero OA. The loss of endothelium-dependent vascular relaxation in hypertension. *Hypertension*. 1986; 8(suppl II):II-61-II-66.
10. Lüscher TF, Vanhoutte PM. Endothelium-dependent contraction to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension*. 1986;8:344-348.
11. Diederich D, Yang A, Bühler FR, Lüscher TF. Impaired endothelium dependent relaxations in hypertensive resistance arteries involve cyclooxygenase pathway. *Am J Physiol*. 1990;258: H445-H451.
12. Tesfamariam B, Halpern W. Endothelium-dependent and endothelium-independent vasodilation in resistance arteries from hypertensive rats. *Hypertension*. 1988;11:440-444.
13. Boegehold MA. Reduced influence of nitric oxide on arteriolar tone in hypertensive Dahl rats. *Hypertension*. 1992;19:290-295.

14. Nakamura T, Prewitt RL. Alteration of endothelial function in arterioles of renal hypertensive rats at two levels of vascular tone. *J Hypertens*. 1992;10:621-627.
15. Johnson PC. The myogenic response. In: Bohr DF, Somlyo AF, Spark HV, Geiger SR, eds. *Handbook of Physiology*. 2nd ed. Bethesda, Md: American Physiological Society; 1980:409-442.
16. Mellander S. Myogenic mechanisms in local vascular control. *Acta Physiol Scand Suppl*. 1988;133:25-42.
17. Sun D, Messina EJ, Kaley G, Koller A. Characteristics and origin of myogenic response in mesenteric arterioles. *Am J Physiol*. 1992; 263:H1486-H1491.
18. Henriksen O, Skagen K, Amtorp O, Hartling O. Augmented vasoconstrictor response to changes in vascular transmural pressure in patients with essential arterial hypertension. *Acta Physiol Scand*. 1981;112:323-329.
19. Osol G, Halpern W. Myogenic properties of cerebral blood vessels from normotensive and hypertensive rats. *Am J Physiol*. 1985;249: H914-H921.
20. Sun D, Kaley G, Koller A. Role of endothelium in function of isolated arterioles of rat mesentery and gracilis muscle. Endothelium. *J Endothelial Cell Res*. 1993;1:115-122.
21. Koller A, Sun D, Kaley G. Role of shear stress and endothelial prostaglandins in flow and viscosity induced dilation of arterioles in vitro. *Circ Res*. 1993;72:1276-1284.
22. Harder DR. Pressure induced myogenic activation of cat cerebral arteries is dependent on intact endothelium. *Circ Res*. 1987;60: 102-107.
23. Harder DR, Sanchez-Ferrer C, Kauser K, Stekiel WJ, Rubanyi GM. Pressure releases a transferrable endothelial contractile factor in cat cerebral arteries. *Circ Res*. 1989;65:193-198.
24. Furchgott RF. Role of endothelium in response of vascular smooth muscle. *Circ Res*. 1983;53:557-573.
25. Vanhoutte PM. Endothelium and control of vascular function: state of the art lecture. *Hypertension*. 1989;13:658-667.
26. Ogletree ML, Harris DN, Greenberg R, Haslanger MF, Nakane M. Pharmacological actions of SQ29548, a novel selective thromboxane antagonist. *J Pharmacol Exp Ther*. 1985;234:435-441.
27. Ku EC, McPherson SE, Signor C, Chertock H, Cash WD. Characterization of imidazo[1,5-a]pyridine-5-hexanoic acid (CGS 13080) as a selective thromboxane synthetase inhibitor using in vitro and in vivo biochemical models. *Biochem Biophys Res Commun*. 1983;112:899-906.
28. Halpern W, Mongeon SA, Root DT. Stress, tension, and myogenic aspects of small isolated extraparenchymal rat arteries. In: Stephens JL, ed. *Smooth Muscle Contraction*. New York, NY: Marcel Dekker, Inc; 1984:427-456.
29. Bohlen HG, Gore RW, Hutchins PM. Comparison of microvascular pressure on normal and spontaneously hypertensive rats. *Microvasc Res*. 1977;13:125-130.

30. DeLano FA, Schmid-Schönbein GW, Skalak TC, Zweifach BW. Penetration of the systemic blood pressure into the microvasculature of rat skeletal muscle. *Microvasc Res*. 1991;41:92-110.
31. Jameson M, Dai FX, Lüscher T, Skopec J, Diederich A, Diederich D. Endothelium-derived contracting factors in resistance arteries of young spontaneously hypertensive rats before development of overt hypertension. *Hypertension*. 1993;21:280-288.
32. Schmid-Schönbein GW, Zweifach BW, DeLano FA, Chen PCY. Microvascular tone in skeletal muscle of spontaneously hypertensive rats. *Hypertension*. 1987;9:164-171.
33. Pagano PJ, Lin L, Sessa WC, Nasjletti A. Arachidonic acid elicits endothelium-dependent release from the rabbit aorta of a constrictor prostanoid resembling prostaglandin endoperoxides. *Circ Res*. 1991;69:396-405.
34. Hayashi K, Epstein M, Loutzenhiser R. Enhanced myogenic responsiveness of renal interlobular arteries in spontaneously hypertensive rats. *Hypertension*. 1992;19:153-160.
35. Takenaka T, Forster H, DeMicheli A, Epstein M. Impaired myogenic responsiveness of renal microvessels in Dahl salt-sensitive rats. *Circ Res*. 1992;71:471-480.
36. Messina EJ, Weiner R, Kaley G. Arteriolar reactive hyperemia: modification by inhibitors of prostaglandin synthesis. *Am J Physiol*. 1977;232:H571-H575.
37. Koller A, Kaley G. Prostaglandins mediate arteriolar dilation to increased blood flow velocity in skeletal muscle microcirculation. *Circ Res*. 1990;67:529-534.
38. Myers TO, Messina EJ, Rodrigues AM, Gerritsen ME. Altered aortic and cremaster muscle prostaglandin synthesis in diabetic rats. *Am J Physiol*. 1985;249:E374-E379.
39. Lukacsko P, Messina EJ, Kaley G. Reduced hypotensive action of arachidonic acid in the spontaneously hypertensive rat. *Hypertension*. 1980;2:657-663.
40. Fu-Xiang D, Skopec J, Diederich A, Diederich D. Prostaglandin H₂ and thromboxane A₂ are contractile factors in intrarenal arteries of spontaneously hypertensive rats. *Hypertension*. 1992;19:795-798.
41. Auch-Schwelk W, Katusic ZS, Vanhoutte PM. Thromboxane A₂ receptor antagonists inhibit endothelium-dependent contractions. *Hypertension*. 1990;15:699-703.
42. Kato T, Iwama Y, Okumura K, Hashimoto H, Ito T, Satake T. Prostaglandin H₂ may be the endothelium-derived contracting factor released by acetylcholine in the aorta of the rat. *Hypertension*. 1990;15:475-481.
43. Mayhan WG. Role of prostaglandin H₂-thromboxane A₂ in responses of cerebral arterioles during chronic hypertension. *Am J Physiol*. 1992;262:H539-H543.
44. Shepherd JT. Circulation to skeletal muscle. In: Shepherd JT, Abboud FM, eds. *Handbook of Physiology, Section 2: The Cardiovascular System, Volume III, Peripheral Circulation and Organ Blood Flow*. Baltimore, Md: Williams & Wilkins Co; 1983:319-370.