

Flow Modulation of Pressure-sensitive Tone in Rat Pial Arterioles

Role of the Endothelium

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Background: **Cerebral arteriolar tone is modulated in response to changes in transmural pressure and luminal flow. The effect of flow on the relation between pressure and diameter has not been fully evaluated in these vessels. This study was conducted to investigate this interaction and to determine the role of the endothelium in mediating it.**

Methods: **Rat pial arterioles from the territory of the posterior cerebral artery were mounted in a perfusion myograph. In some arterioles, the endothelium was removed by air perfusion. Diameters were recorded at pressures from 20 to 200 mmHg in the presence and absence of flow (10 μl/min). The response to flow (0–30 μl/min) was recorded at 60 and 120 mmHg.**

Results: **In the absence of flow, endothelium-intact arterioles demonstrated tone at distending pressures between 40 and 140 mmHg. In the presence of flow, tone did not develop until pressure exceeded 100 mmHg, and the vessels remained active at pressures up to 200 mmHg. Endothelium-denuded arterioles developed tone at the same pressure when perfused as when unperfused, but perfused vessels were able to maintain active tone at higher pressures. At 60 mmHg, flow caused dilation if the endothelium was intact and constriction if it had been removed. At 120 mmHg, flow caused constriction. Endothelium-dependent flow–relaxation was inhibited by N^G-nitro-L-arginine methyl ester (10^{-5} M) and abolished by indomethacin (10^{-5} M).**

Conclusion: **Flow inhibits the development of pial arteriolar tone at low intraluminal pressures through endothelium-dependent mechanisms. Conversely, perfusion extends the upper limit of the myogenically regulated pressure range through endothelium-independent activation of arteriolar smooth muscle contraction. (Key words: Autoregulation; cerebral blood flow; myogenic response; nitric oxide; prostaglandins.)**

THE capacity of pial arterioles to adjust their tone in response to fluctuations in distending pressure contributes to autoregulation of cerebral blood flow.[1–3] In most vascular beds, flow activates the release of endothelium-derived relaxing factors that oppose pressure-sensitive (myogenic) vasoconstriction and prevent it from overriding metabolic regulatory mechanisms.[4,5] In cerebral arterial segments, in contrast, the response to flow changes to constriction as basal tone is increased,[6–9] suggesting that, in this circulation, flow may enhance rather than oppose myogenic tone. However, the maintenance of an established level of tone involves different pathways of smooth muscle activation than does contraction evoked by an increase in transmural pressure.[10] Therefore, the effect of basal tone on the flow–response and the effect of flow on myogenic contraction represent interactions between different mechanisms, and the hypothesis that luminal flow enhances the pressure-sensitive constriction needs to be specifically tested.

Factors that modulate pressure-sensitive tone may also alter the range of pressures over which the myogenic response is active.[11–14] A change in the threshold pressure for arteriolar contraction would affect the capacity to maintain perfusion at low arterial pressures. Conversely, arterial pressures greater than that at which active tone is lost are associated with hemorrhagic stroke and hypertensive encephalopathy.[15–17] Despite their relevance, the effects of luminal flow on the upper and lower limits of the myogenically regulated pressure range have not been assessed in cerebral arterioles. Accordingly, this study was conducted to determine the effect of perfusion on the relation between transmural pressure and diameter in rat pial arterioles and the role of the endothelium in mediating this interaction.

Methods and Materials

Arteriole Isolation

The protocol was approved by the animal care committee of the Montreal Neurological Institute, Montreal, Quebec, Canada. Male Sprague-Dawley rats (200–250 g) were stunned and then killed by decapitation. The entire brain was immediately removed and placed in a silicone-lined dissecting dish containing cold (0–4°C), oxygenated, bicarbonate-buffered physiologic salt solution (PSS; NaCl 119 mM; KCl 4.7 mM, KH_2PO_4 1.18 mM; $MgSO_4$ 1.17 mM, $NaHCO_3$ 24.9 mM, EDTA 400 μM; $CaCl_2$ 3.7 mM, glucose 5 mM, pH 7.4). The posterior cerebral artery was followed until an unbranched arteriolar segment at least 1 mm in length could be identified. The segment was dissected free, cleared from the adhering tissue, and transferred to a plexiglas vessel chamber (Living Systems, Burlington, VT) containing PSS. Inflow and outflow micropipettes were matched for resistance to flow, and the system was arranged to have mirror symmetry with the axis of symmetry located at the middle of the arteriolar segment. This resulted in equal resistances of

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the two sides of the system (from pressure transducer to the tip of the pipette). The proximal end of the arteriole was mounted to the inflow cannula and secured with 12-0 suture. The perfusion pressure was then increased to 20 mmHg with a pressure-servo micropump system (Living Systems), taking its inflow from a reservoir of PSS. After the arteriole was cleared of clotted blood, its distal end was mounted to the outflow cannula. Both the inflow and outflow cannulae were connected to microflow pumps (Living Systems). The inflow pump was a constant flow pump that had been calibrated over a range of flows from 0 to 45 μl/min, whereas the outflow pump was regulated by a servo system to maintain a constant downstream pressure. This allowed the vessel to be perfused at constant flow set by the upstream pump while the midpoint pressure (calculated as the average of the upstream and downstream pressures) was maintained at the desired level by adjusting the pressure target for the servo mechanism regulating the downstream pump. In this way, flow is established by changing proximal and distal pressures by an equal amount but in opposite directions such that the average of the upstream and downstream pressures (midpoint luminal pressure) remains constant.[18,19] In preliminary studies, we measured midpoint intraluminal pressure by micropuncture using a servo-null pressure transducer (micropressure system model 900; World Precision Instruments, Sarasota, FL) during incremental pressure changes from 0 to 100 mmHg (the range of the pressure transducer) at each of the flows used in the current study. As reported previously by other investigators,[18,19] we found no difference between the average of upstream and downstream pressures and the directly measured midpoint pressure at any of the flow rates studied. The arteriole was set to its *in situ* length using an eyepiece micrometer. The inflow cannula was closed, and the transmural pressure (*i.e.*, intraluminal pressure relative to atmospheric pressure) was slowly increased to 60 mmHg at zero flow by adjusting the downstream pressure target for the servo mechanism regulating the outflow pump. The pressure–servo system was then placed in manual mode, where a stable pressure value indicated that there was no leak in the system. Vessels in which a leak was detected were discarded.

The apparatus was transferred to an inverted microscope (Nikon TMS-F, 20× objective; Nikon, Melville, NY). Steady state measurements of internal diameter at the midpoint of the arteriolar segment were made using a high resolution CCD video camera (Hitachi KPC503; Hitachi, San Jose, CA) and a video calliper (Living Systems) calibrated using a stage micrometer. The vessel was continuously superfused with PSS flowing through the chamber at a rate of 6 ml/min. The chamber was warmed to 37°C using a heat exchanger in line with the superfusion pump over 60 min and maintained at this temperature throughout the experimental protocol. Chamber temperature and pH were monitored continuously using a probe (Oakton series 35616, Singapore) and samples of the superfusing buffer were periodically drawn from the chamber for gas analysis (model 995, AVL Instruments, Graz, Austria). A plexiglas cover excluded ambient air from the chamber. The reservoir containing the superfusate and the vessel chamber itself were bubbled with gas, the composition of which was adjusted using separate tanks and regulators for each of oxygen, carbon dioxide, and nitrogen, to achieve oxygen and carbon dioxide partial pressure values in the vessel chamber of 100 mmHg and 40 mmHg, respectively. Under these conditions, the vessels gradually developed spontaneous tone independent of vasoconstrictor agents. Endothelium-dependent and -independent dilation was evaluated in all vessels by determining the ability of acetylcholine (10^{-5} M, Sigma) and diethylamine–nitric oxide (DEA-NO, 10^{-4} M, Research Biochemicals International, Nantick, MA), respectively, to inhibit intrinsic tone. On completion of each experiment, the internal diameter was recorded with the vessels in the passive state at a distending pressure of 60 mmHg. The passive state was achieved by bathing the arterioles in calcium-free PSS containing EGTA (4 mM) and adenosine (10^{-4} M).

Endothelial Removal

In vessels in which the endothelium was to be removed, the responses to acetylcholine and DEA-NO were evaluated after the initial equilibration period as previously described. The intraluminal pressure was then reduced to 20 mmHg, the stopcock on the outflow cannula was opened, and the arterioles were perfused with 2 ml air. The arterioles were then perfused with PSS for 10–15 min at 20 mmHg to flush the separated endothelial layer from the vessel lumen and out of the cannula system. The outflow cannula was closed, the intraluminal pressure was restored to 60 mmHg, and the dilatory responses to acetylcholine and DEA-NO were once again determined. In a previous histologic study,[20] elimination of vasodilation in response to acetylcholine with an intact vasodilatory response to nitric oxide donors after this procedure has been shown to be associated with ablation of the endothelial cell layer and, in the current study, was taken as evidence of successful endothelial removal.

Protocols

Three protocols were undertaken to determine (1) the effect of perfusion on the steady state pressure–diameter relation; (2) the effect of changing transmural pressure on the response to flow; and (3) the roles of nitric oxide and prostaglandin release in mediating flow–dilation.

Isoflow Pressure–Diameter Relations. In separate groups of arterioles, the relation between ID and intraluminal pressure was recorded during zero flow pressur-

ization (n = 14) and during perfusion with the upstream pump set at a constant flow rate of 10 μl/min (n = 14). In half of the arterioles the endothelium was removed. After recording steady state diameter at 60 mmHg, the intraluminal pressure was stepped upward or downward to present pressures at intervals of 20 mmHg over a range from 20 to 140 mmHg in random order. Because, in preliminary studies, arterioles that stabilized at 60 mmHg and pressurized above 140 mmHg in the absence of luminal flow, dilated and did not regain their previous level of spontaneous tone when returned to the lower pressure, pressures greater than 140 were presented last. The highest intraluminal pressures that could be achieved with this system were 200 mmHg in the absence of flow and 180 mmHg during perfusion at 10 μl/min. Diameter was measured 10 min after each pressure change, at which time the vessel had reached steady state. On completion of the active measurements, the superfusion solution was changed to Ca^{2+}-free PSS containing EGTA (4 mM) and adenosine (10^{-4} M), and pressure–diameter data were collected for the vessel in the passive state.

A separate group of endothelium-intact (n = 6) and endothelium-denuded (n = 6) arterioles was equilibrated for 1 h at 37°C and a distending pressure of 60 mmHg. Perfusion at 10 μl/min was then initiated. After recording the ID under these conditions, intraluminal pressure was slowly increased to 180 mmHg by adjusting the upstream and downstream pressures as previously described. The internal diameter at this pressure was recorded, and then the perfusion pump was stopped. The diameter was again recorded after the vessels had reached steady state at 180 mmHg in the absence of flow. On completion of each experiment, the passive diameter was recorded under each of the experimental conditions.

Isopressure Flow–Diameter and Shear Stress–Diameter Relations. In separate groups of arterioles (n = 14 per group), perfusate flow was increased from 0 to 30 μl/min in 5-μl/min steps while maintaining the intraluminal pressure at either 60 or 120 mmHg (*i.e.*, near the lower and upper ends of the plateau phase of the pressure–diameter relation). In half of the arterioles in each group, the endothelium was removed. Diameter was recorded after the vessel had reached steady state, 10 min after each flow step. On completion of each experiment, the passive diameter at 60 mmHg was recorded. As described previously, the passive state was achieved by bathing the arterioles with Ca^{2+}-free PSS containing EGTA and adenosine. Because diameter is normally regulated to preserve shear stress at the endothelial–luminal interface within a narrow range,[18,21,22] it has been argued that shear, not flow, is the stimulus for endothelial mediator release and should be plotted as the independent variable. Accordingly, the relation between shear stress and steady state diameter after each flow step was also plotted. Shear stress (τ) was calculated as: $\tau = 4\eta Q/\pi r^3$, where η is viscosity (Poises), Q is flow (milliliters per second), and r is vessel radius (centimeters). Viscosity of the perfusate (PSS), measured against water in a viscometer (Cannon Instrument Co., State College, PA) at 37°C was 0.0071 Poises.

Effect of Indomethacin and N^G-nitro-L-arginine Methyl Ester on Flow–Dilation. The effect of the nitric oxide synthase antagonist N^G-nitro-L-arginine methyl ester (L-NAME) and of indomethacin on the dilatory response to acetylcholine, DEA-NO, and prostaglandin E_2 (PGE_2), were evaluated in endothelium-intact vessels. This was performed to determine if differences in responsiveness to flow after treatment with these agents could be attributed to nonspecific effects.[23] At the end of the stabilization period, the responses to acetylcholine (10^{-5} M), DEA-NO (10^{-4} M), and PGE_2 (10^{-9} M) were determined at a distending pressure of 60 mmHg in the absence of luminal flow by infusing these agents into the superfusion line using the syringe pump. The infusion was stopped, and after the arterioles had returned to their baseline diameters, L-NAME (10^{-5} M, n = 7) or indomethacin (10^{-5} M,[24] n = 7) was added to the superfusion solution. After a further 15-min equilibration period, the responses to acetylcholine, DEA-NO, and PGE_2 were again recorded.

In separate groups of arterioles, at the end of the stabilization period and after evaluating the responses to acetylcholine and DEA-NO, indomethacin (10^{-5} M, n = 7), L-NAME (10^{-5} M, n = 7), or both (n = 7) were added to the superfusion and perfusion solutions. After a further 15-min equilibration period, the flow–diameter relation was determined at distending pressure of 60 mmHg.

Statistical Analysis

Differences among multiple means were detected by analysis of variance corrected for repeated measures where appropriate and analyzed *post hoc* using the Student-Neuman-Keuls procedure. Unless otherwise stated, data are presented as mean ± SD in n number of animals with *P* less than 0.05 representing statistical significance.

Results

The IDs for all vessels studied averaged 88 ± 11 μm at the end of the equilibration period. This value is less than the diameter recorded under passive conditions at an intraluminal pressure of 60 mmHg (152 ± 13 μm), indicating that by the end of the equilibration period the arterioles had developed spontaneous tone. The average diameters during relaxation with acetylcholine (129 ± 11 μm) and DEA-NO (149 ± 12 μm) were also significantly greater than that at the end of the equilibration period. The diameter during treatment with DEA-NO did not differ from that recorded in the passive condition.

Pressure–Diameter Relations

Figure 1 illustrates the relation between intraluminal pressure and ID for endothelium-intact and -removed vessels under active and passive conditions in the absence of luminal flow. Arterioles developed tone at intraluminal pressures greater than 40 mmHg, as evidenced by a significant difference in their diameter at this pressure compared with their corresponding passive diameter. At pressures exceeding 140 mmHg, the vessels dilated toward the passive relation. Results obtained in arterioles from which the endothelium was removed did not differ from those obtained in vessels in which the endothelium was intact.

The top of figure 2 presents the pressure–diameter relations for endothelium-intact and -removed vessels that were perfused at 10 μl/min. In the presence of flow, endothelium-intact arterioles dilated passively as intraluminal pressure was increased until it exceeded 100 mmHg. Above this level, further increase in pressure elicited constriction. The perfused vessels were able to maintain a constant diameter at pressures at which arterioles pressurized in the absence of flow were forced to dilate. Endothelium-removed arterioles perfused at 10 μl/min developed tone at pressures above 40 mmHg, and the vessels continued to demonstrate active tone at the highest pressure studied. The effect of stopping flow on the ID of vessels perfused at 10 μl/min at a distending pressure of 180 mmHg is illustrated in the bottom of figure 2. Consistent with the results illustrated in the top of figure 2, cessation of flow was associated with dilation.

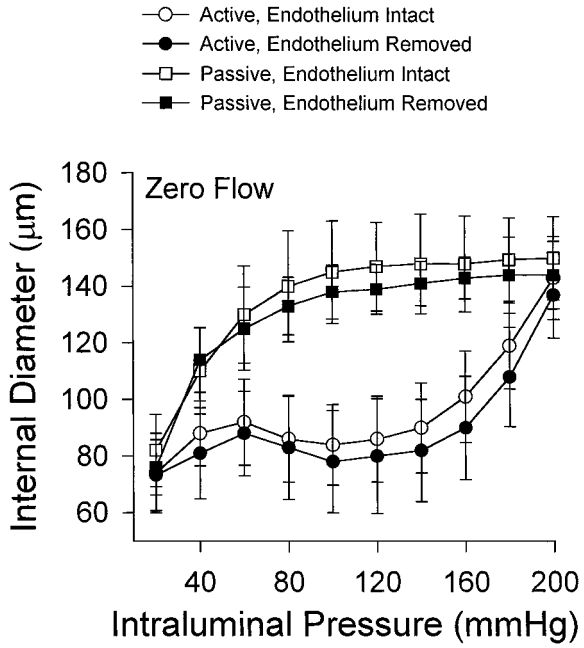

Fig. 1. Relations between intraluminal pressure and ID for endothelium-intact and -removed arterioles studied in the absence of luminal flow. Active denotes arterioles that demonstrate spontaneous tone. Passive indicates that tone has been eliminated by superfusion with Ca^{2+}-free buffer containing 10^{-4} M adenosine. $P < 0.05$ for difference between active and corresponding passive diameters in both endothelium-intact and -removed vessels. $P > 0.05$ for differences between endothelium-intact and -removed vessels under both active and passive conditions.

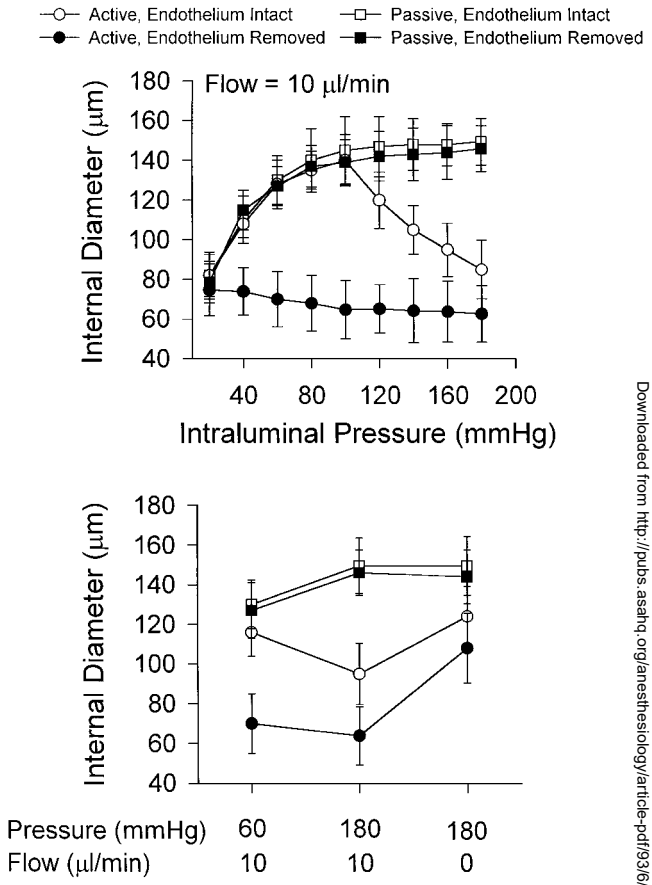

Fig. 2. (*Top*) Active and passive pressure–diameter relations for endothelium-intact and -removed arterioles in the presence of luminal flow at 10 μl/min. $P < 0.05$ for differences between active and passive conditions for both endothelium-intact and -removed arterioles. $P < 0.05$ for difference between active endothelium-intact and active endothelium-removed vessels. $P < 0.05$ for differences between active pressure–diameter relations in perfused arterioles and those studied at zero flow (illustrated in fig. 1). (*Bottom*) Active and passive diameters of endothelium-intact arterioles at distending pressures of 60 and 180 mmHg in the presence and absence of luminal flow (10 μl/min). *$P < 0.05$ for difference between active and passive diameters.

Flow–Diameter and Shear Stress–Diameter Relations

The top of figure 3 illustrates the relations between flow and diameter and between shear stress and diameter in endothelium-intact and -removed arterioles at an intraluminal pressure of 60 mmHg. In endothelium-intact vessels, pressurized to 60 mmHg, increases in flow and shear stress elicited dilation with diameters approaching those recorded under passive conditions at flow rates greater than 20 μl/min. In endothelium-removed arterioles pressurized to 60 mmHg, increasing flow and shear stress elicited vasoconstriction.

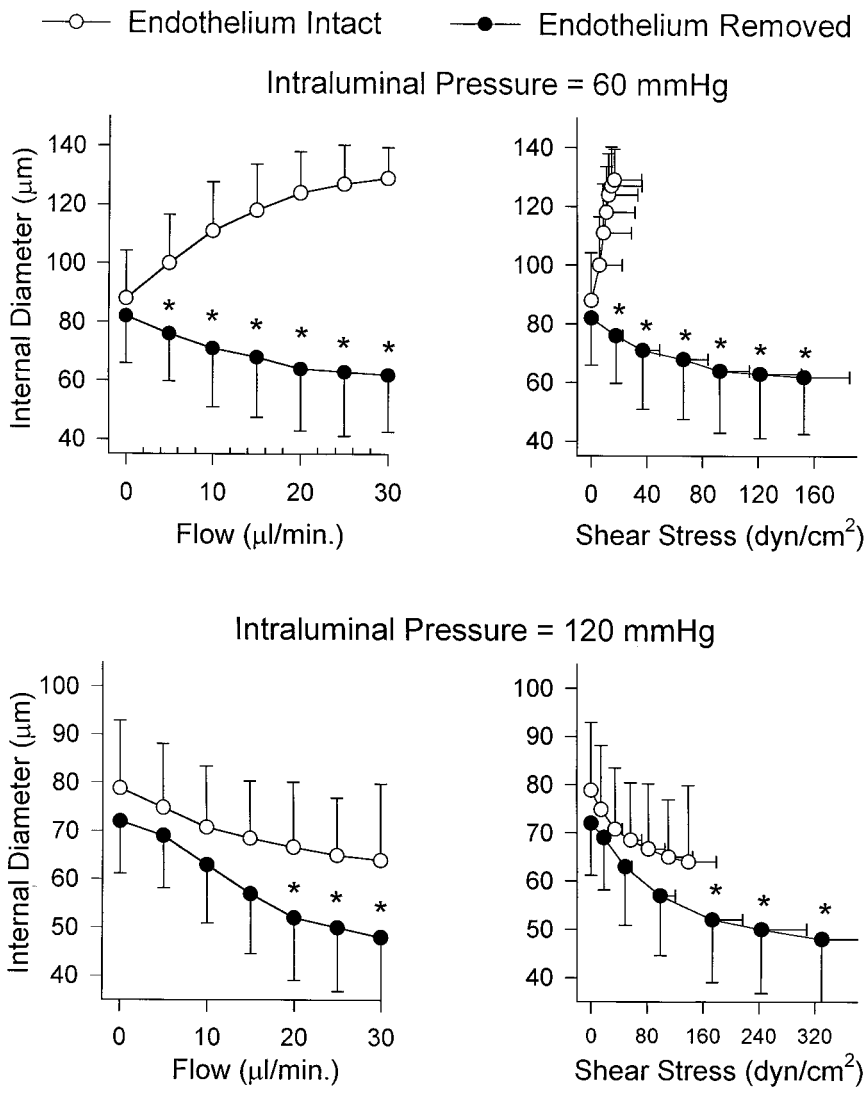

Fig. 3. (*Top*) Flow–diameter and shear stress–diameter relations for endothelium-intact and -removed arterioles at an intraluminal pressure of 60 mmHg. *$P < 0.05$ for difference between endothelium-intact and -removed groups. (*Bottom*) Flow–diameter and shear stress–diameter relations for endothelium-intact and -removed arterioles at an intraluminal pressure of 60 mmHg. *$P < 0.05$ for difference between endothelium-intact and -removed groups.

The bottom of figure 3 presents the results obtained in endothelium-intact and -removed arterioles studied at an intraluminal pressure of 120 mmHg. At this level of intraluminal pressure, increases in flow and shear stress are associated with vasoconstriction that is more pronounced in vessels from which the endothelium had been removed.

Effect of Indomethacin and N^G*-nitro-L-arginine Methyl Ester on Flow–Dilation*

Table 1 presents the effects of L-NAME (10^{-5} M) and indomethacin (10^{-5} M) on the change in diameter (response in micrometers) of endothelium-intact vessels (intraluminal pressure = 60 mmHg) after addition of acetylcholine, PGE_2, and DEA-NO to the superfusion

Table 1. Effects of L-NAME and Indomethacin on Responses to Acetylcholine DEA/NO and Prostglandin E_2

	Acetylcholine		DEA/NO		Prostaglandin E_2	
	Baseline (μm)	Response (μm)	Baseline (μm)	Response (μm)	Baseline (μm)	Response (μm)
Control (n = 14)	90 ± 12	35 ± 8	94 ± 14	61 ± 10	94 ± 11	42 ± 7
L-NAME (n = 7)	86 ± 10	7 ± 6*	84 ± 12	58 ± 11	92 ± 10	39 ± 6
Indomethacin (n = 7)	88 ± 9	32 ± 6	90 ± 10	62 ± 13	90 ± 12	34 ± 8

* $P < 0.05$ for difference from the response in the absence of antagonists (control).

NAME = N^G-nitro-L-arginine methyl ester; DEA/NO = diethylamine–nitric oxide.

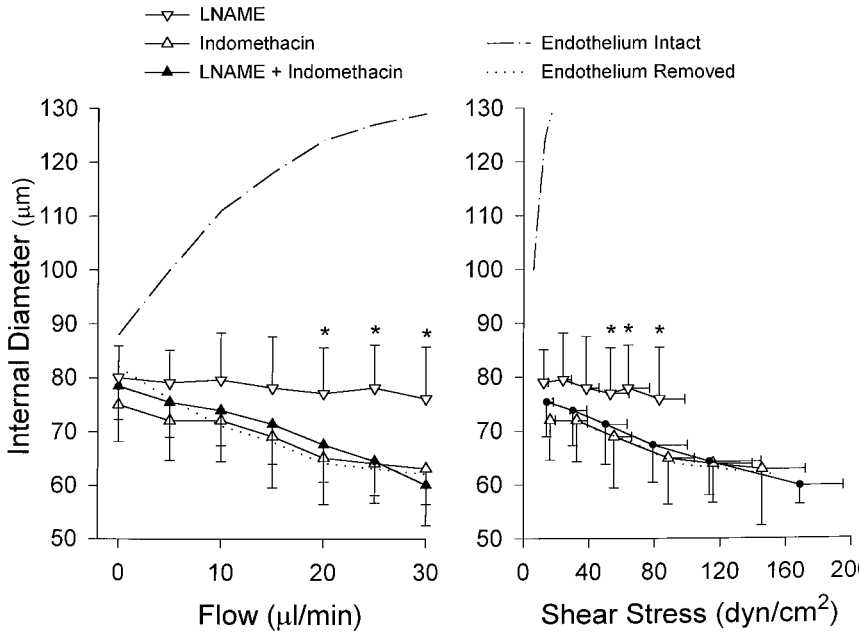

Fig. 4. Effect of treatment with indomethacin (10^{-5} M), N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-5} M) and with both indomethacin and L-NAME on the relations between flow and ID and between shear stress and ID in endothelium-intact arterioles at an intraluminal pressure of 60 mmHg. $P < 0.05$ for differences between untreated endothelium-intact arterioles and arterioles treated with L-NAME, indomethacin, and with both indomethacin and L-NAME. $P > 0.05$ for difference between arterioles treated with indomethacin and arterioles treated with both indomethacin and L-NAME. *$P < 0.05$ for difference from endothelium-removed vessels. Lines representing responses in endothelium-intact and -removed untreated arterioles (complete data in fig. 2) are included for reference.

solution. L-NAME inhibited dilation to acetylcholine without significantly altering the responses to PGE_2 or DEA-NO. Indomethacin in this concentration had no effect on the responses to acetylcholine, PGE_2, or DEA-NO.

Figure 4 illustrates the effect of treatment with indomethacin, with L-NAME, and with both agents on diameter during increases in flow and shear stress in endothelium-intact arterioles at an intraluminal pressure of 60 mmHg. Lines representing the results obtained in untreated endothelium-intact and -removed arterioles (presented in fig. 2) are included in figure 3 for reference. L-NAME attenuated dilation in response to increases in flow and shear stress ($P < 0.05$ for difference from untreated endothelium-intact arterioles) but did not reproduce the effect of endothelial ablation. Responses in endothelium-intact arterioles treated with indomethacin or with both indomethacin and L-NAME did not differ from those in arterioles from which the endothelium had been removed.

Discussion

The main findings of this study are that, in rat pial arterioles, (1) the transmural pressure required for the development of active tone is higher in the presence than in the absence of luminal flow; (2) the upper limit of the myogenically regulated pressure range is higher in perfused than in unperfused vessels; (3) flow elicits endothelium-dependent dilation at low intraluminal pressures (60 mmHg) and endothelium-independent constriction at high intraluminal pressures (120 mmHg); and (4) both L-NAME and indomethacin inhibit flow dilation in vessels pressurized to 60 mmHg, and the effect of indomethacin alone or in combination with L-NAME is similar to that of endothelial ablation.

This is the first study to assess the effect of perfusion on the entire steady state pressure–diameter relation in pial arterioles. The vessels selected for study were from the territory of the posterior cerebral artery from which blood flow to the occipital lobes is derived. These were chosen for their physiologic relevance in the regulation of cerebral blood flow and microvascular pressure[25,26] and because previous studies have indicated that they demonstrate a high level of basal pressure-sensitive tone at intraluminal pressures within the physiologic range.[2] However, it should be recognized that the intrinsic and neurohumoral mechanisms that regulate tone vary markedly between pial and intraparenchymal arterioles[28,29] between arterioles from different regions of the brain[29] and among vessels of different size.[30] This heterogeneity limits the ability to generalize conclusions based on our findings to other components of the cerebral vasculature.

In the absence of luminal flow, the vessels in the current study maintained a relatively constant diameter over a range of transmural pressures from 40 to 140 mmHg. A similar pressure range has been reported in porcine coronary arterioles[25] and in arterioles isolated from the rat diaphragm,[32,33] cremaster muscle,[34] and mesentery.[35] In accord with these previous reports and with results obtained in some,[36] but not all,[37,38] large artery preparations, this response does not require the presence of an intact endothelial cell layer.

In endothelium-intact pial arterioles perfused at a constant rate of 10 μl/min, we found that the part of the pressure–diameter relation less than 100 mmHg resembles the passive curve. At pressures greater than 100 mmHg, perfused vessels developed active tone that persisted at the highest pressure studied (180 mmHg). In contrast, endothelium-removed arterioles perfused at 10 μl/min developed tone at a distending pressure sim-

ilar to that recorded in the absence of flow. As in endothelium-intact vessels, perfused arterioles from which the endothelium had been removed maintained their level of tone as transmural pressure was increased to levels above that at which, in the absence of flow, unperfused vessels were forced to dilate.

In previous studies, partial pressure–diameter relations have been generated in the presence of luminal flow in porcine coronary arterioles,[39] rabbit renal afferent arterioles,[40] and rat intracerebral arterioles.[41] Flow was found to inhibit tone at low pressures in those studies and, as a result, the lower end of the pressure–diameter relation was shifted upward. Therefore, under physiologic conditions (*i.e.*, in the presence of luminal flow), the endothelium plays an important role in determining the pressure threshold for arteriolar smooth muscle activation in both cerebral and noncerebral systemic circulations.

The effect of perfusion on the upper limit of the myogenically regulated pressure range has not previously been assessed in arterioles from any vascular bed. Our current results indicate that, in pial arterioles, flow provides an additional endothelium-independent stimulus for contraction and permits diameter to be maintained in the face of high distending pressures. This may protect the vessel from the deleterious effects of pressurization by preventing a disproportionate increase in wall tension.

In arterioles in which flow was increased while intraluminal pressure was maintained at 60 mmHg, perfusion was accompanied by dilation, but tone was not eliminated (diameter did not equal that recorded under passive conditions) until flow exceeded 20 μl/min. This is at variance with our observation that, in arterioles in which pressure–diameter relations were generated, tone is completely suppressed by perfusion at 10 μl/min at pressures less than 100 mmHg. The difference in the results of the two protocols suggests that flow is less effective at inhibiting preexisting arteriolar tone than it is in preventing its development after changes in transmural pressure. In skeletal muscle arterioles, the inhibitory effect of L-type Ca^{2+} channel antagonists on the response to changes in distending pressure was found to differ from that on basal tone.[10] Therefore, the mechanisms that mediate myogenic responses and those that maintain the existing level of tone appear to involve different pathways of Ca^{2+} entry. Differences in the susceptibility of these pathways to inhibition by endothelium-derived mediators could account for the dissimilarity in the effect of flow between the two protocols in the current study.

Flow has been shown to elicit vasodilation, which is eliminated by endothelial ablation, in large arteries[42,43] and in isolated arterioles[19,44] from a number of vascular beds. In previous studies conducted in the cerebral vasculature, branches of the middle and posterior cerebral arteries of the rabbit were found to relax in response to perfusion when the intraluminal pressure was less than 60 mmHg.[6,45] Flow–dilation was attenuated but not eliminated after removal of the endothelium, indicating that a mechanism exists by which flow may trigger vascular smooth muscle relaxation directly. However, this response may be species-specific rather than a characteristic of the cerebral circulation, because similar results have been obtained in arterial segments from other vascular beds in the rabbit.[46] In rat intracerebral arterioles, Ngai and Winn[41] found that at a constant transmural pressure of 60 mmHg, flows less than 10 μl/min elicit dilation, whereas higher perfusion rates do not. The flow response in endothelium-removed vessels was not investigated in that study. Our current results indicate that in rat pial arterioles, at an intraluminal pressure of 60 mmHg, flows up to 30 μl/min elicit dilation, with the result that shear stress remained constant. Flow–dilation, and the tight regulation of shear stress demonstrated in endothelium-intact vessels, was eliminated after endothelial ablation. We conclude that the mechanisms that mediate flow–dilation in these vessels are localized to the endothelium.

In previous studies, Gaw and Bevan[45] and Thorin-Trecases and Bevan[6] showed that antagonists of nitric oxide synthesis inhibit flow relaxation in endothelium-intact but not endothelium-removed rabbit cerebral artery segments. Similarly, Ngai and Winn[41] and Shimoda *et al.*[9] found that L-arginine analogs eliminate flow–dilation in rat intracerebral arterioles and piglet cerebral arteries, respectively. In the latter study, indomethacin had no effect on flow–dilation, and the investigators concluded that, in the cerebral vasculature in these species, this response is entirely mediated by nitric oxide. In contrast, in arterioles from the rat cremaster muscle, indomethacin was found to eliminate flow dilation[18] and, although the effect of inhibiting nitric oxide synthesis was not evaluated, it was concluded that the response was entirely mediated by endothelial prostaglandin release. All of these previous results are at variance with the findings of Koller *et al.*,[47] who studied the effects of indomethacin and N^G-nitro-[scap]l-arginine on flow–dilation in arterioles from the rat gracilis muscle. In that study, these antagonists each reduced flow dilation,[3] but simultaneous treatment with both agents was required to abolish the response, indicating that the interaction between nitric oxide and cyclooxygenase pathways is additive. The present results demonstrate that, in arterioles from the rat pial circulation, both nitric oxide and prostaglandin release must be intact for the dilatory response to occur. Our finding that treatment with L-NAME blocked flow–dilation even though inhibition of prostaglandin synthesis (with indomethacin) was equivalent to endothelial ablation suggests that, in this circulation, the interaction between these two pathways is not simply additive, as has been reported in arterioles isolated from

gracilis muscle.[47] This result is not caused by a nonspecific effect of L-NAME on prostaglandin-mediated relaxation because, in the concentrations used, this agent had no effect on the dilator response to PGE_2. Administration of exogenous nitric oxide has been shown to modulate prostaglandin synthesis in several cell types,[48,49] and activation of cyclooxygenase by endogenously produced nitric oxide has been demonstrated in cultured endothelial cells.[50] A permissive role for nitric oxide in pial arterioles, such that its production within the endothelial cell is required for full activation prostaglandin release by flow, would provide an explanation for our results. Further studies are required to evaluate this hypothesis.

Perfusion has been previously reported to elicit constriction in rabbit mesenteric,[51] femoral,[52] and cerebral arteries,[6,7] piglet cerebral arteries,[9] and cat pulmonary arteries.[53] In rabbit femoral arteries, preconstriction did not affect this response.[52] In contrast, rabbit cerebral arteries demonstrate endothelium-dependent flow–dilation at low levels of basal tone and endothelium-independent flow–constriction when tone is enhanced either by increasing transmural pressure[6,7,9,54] or with agonists.[6,55] In porcine coronary arterioles, increasing basal tone inhibits flow–dilation[39,56] but has not been shown to reverse the response from dilation to constriction as it does in the cerebral circulation. The results of the present study indicate that rat pial arterioles resemble qualitatively rabbit cerebral arteries in that the direction of the flow response depends on whether transmural pressure, and hence basal myogenic tone, exceeds a certain threshold. The threshold pressure for reversal of the response was 100 mmHg in the arterioles used in the current study compared with 60 mmHg in the larger cerebral arteries studied previously.[6,7,54] This variability could be species-dependent. Nevertheless, it is attractive to speculate that greater sensitivity of the flow response to pressure in larger upstream arteries compared with smaller downstream arterioles would shift the locus of pressure dissipation to more muscular, thicker-walled vessels during episodes of acute arterial hypertension.

Because increasing intraluminal pressure enhances arteriolar tone directly, it is difficult to determine, either from the results of previous studies or from our current findings, the extent to which concomitant impairment of endothelium-derived relaxing factor release contributes to the pressure-dependent reversal of the flow response from dilation to constriction. In the present study, however, we found that at a distending pressure of 120 mmHg, flow–constriction was more marked in endothelium-intact than in endothelium-denuded vessels. Therefore, the endothelium continues to play some role in modulating arteriolar tone at high pressures, either through the release of vasoactive mediators or by providing a physical barrier between the smooth muscle layer and mechanical stresses applied at the luminal surface.

The interaction between the effects of pressure and flow on pial arteriolar tone is inherently protective. In the presence of an intact endothelial cell layer, myogenic tone is eliminated in perfused vessels at low pressures. This is required if cerebral perfusion is to be optimized during episodes of hypotension. Above a critical level, further pressure elevations elicit vasoconstriction, and the capacity to sustain active tone in the face of very high transmural pressures is enhanced. This may be an important mechanism limiting exposure of exchange vessels to the elevated arterial pressure levels that occur during intense muscular contraction, emotional stress, and hypertensive crises.

References

1. Osol G, Halpern W: Myogenic properties of cerebral blood vessels from normotensive and hypertensive rats. Am J Physiol 1985; 249:H914–21
2. Kontos HA, Richardson DW, Patterson JL: Roles of hypercapnia and acidosis in the vasodilator response to hypercapnic acidosis. Am J Physiol 1968; 215: 1406–8
3. Kontos HA, Wei EP, Navari RM, Levasseur JE, Rosenblum WI, Patterson JL: Responses of cerebral arteries and arterioles to acute hypotension and hypertension. Am J Physiol 1978; 234:H371–83
4. Griffith TM, Edwards DH: Myogenic autoregulation of flow may be inversely related to endothelium-derived relaxing factor activity. Am J Physiol 1990; 258: H1171–80
5. Meininger GA, Mack CA, Fehr KL, Bohlen HG: Myogenic vasoregulation overrides local metabolic control in resting rat skeletal muscle. Circ Res 1987; 60:861–70
6. Thorin-Trescases N, Bevan JA: High levels of myogenic tone antagonize the dilator response to flow of small rabbit cerebral arteries. Stroke 1998; 29:1194–1201
7. Garcia-Roldan JL, Bevan JA: Flow-induced constriction and dilation in cerebral resistance arteries. Circ Res 1990; 66:1445–58
8. Bevan JA, Joyce EH: Flow dependent contraction observed in a myograph-mounted resistance artery. Blood Vessels 1988; 25:261–4
9. Shimoda LA, Norins NA, Jeutter DC, Madden JA: Flow-induced responses in piglet isolated cerebral arteries. Pediatr Res 1996; 39:574–83
10. Hill MA, Meininger GA: Calcium entry and myogenic phenomena in skeletal muscle arterioles. Am J Physiol 1994; 267:H1085–92
11. Ward ME, Hussain SNA: Diaphragmatic pressure-flow relationship during haemorrhagic shock: Role of nitic oxide. J Appl Physiol 1994; 77:2244–9
12. Kobari M, Fukuuchi Y, Tomita M, Tanahashi N, Takeda H: Role of nitric oxide in regulation of cerebral microvascular tone and autoregulation of cerebral blood flow in cats. Brain Res 1994; 667:255–62
13. Smith TP, Canty JM: Modulation of coronary autoregulatory responses by nitric oxide: Evidence for flow-dependent resistance adjustments in conscious dogs. Circ Res 1993; 73:232–40
14. Faber JE, Meininger GA: Selective interaction of α-adrenoceptors with myogenic regulation of microvascular smooth muscle. Am J Physiol 1990; 259: H1126–33
15. Tamaki KT, Sadoshima S, Baumbach GL, Iadecola C, Reis DJ, Heistad DD: Evidence that disruption of the blood brain barrier precedes reduction in cerebral blood flow in hypertensive encephalopathy. Hypertension 1984; 6(Suppl 1):I-75–81
16. Skinhoj E, Strandgaard S. Pathogenesis of hypertensive encephalopathy. Lancet 1973; 1:461–2
17. Mackenzie ET, Strandgaard S: Effects of acutely induced hypertension in cats on pial arteriolar caliber, local cerebral blood flow and the blood brain barrier. Circ Res 1976; 39:33–41
18. 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; 72:1276–84
19. Kuo L, Davis MJ, Chilian WM: Endothelium-dependent, flow-induced dilation of isolated coronary arterioles. Am J Physiol 1990; 259:H1063–70
20. Messina EJ, Sun D, Koller A, Wollin MS, Kaley G: Role of endothelium-derived prostaglandins in hypoxia-elicited arteriolar dilation in rat skeletal muscle. Circ Res 1992; 71:790–6
21. Olesenn SP, Clapham DE, Davies PF: Haemodynamic shear stress activates a K+ current in vascular endothelial cells. Nature 1988; 331:168–70

22. Frame MDS, Sarelius IH: Endothelial cell dilatory pathways link flow and wall shear stress in an intact arteriolar network. J Appl Physiol 1996; 81:2105–14
23. Koller A, Sun D, Messina EJ, Kaley G: L-arginine analogues blunt prostaglandin-related dilation of arterioles. Am J Physiol 1993; 264:H1194–9
24. Koller A, Messina EJ, Wolin MS, Kaley G: Endothelial impairment inhibits prostaglandin and EDRF-mediated arteriolar dilation in vivo. Am J Physiol 1989; 257:H1966–70
25. Faraci FM, Kadel KA, Heistad DD: Vascular responses of dura mater. Am J Physiol 1989; 257:H157–61
26. Faraci FM, Heistad DD: Regulation of large cerebral arteries and cerebral microvascular pressure. Circ Res 1990; 66:8–17
27. McCarron JG, Osol G, Halpern W: Myogenic responses are independent of the endothelium in rat pressurized posterior cerebral arteries. Blood Vessels 1989; 26:315–9
28. Faraci FM, Mayhan WG, Heistad DD: Segmental vascular responses to acute hypertension in cerebrum and brain stem. Am J Physiol 1987; 21:H738–42
29. Takayasu M, Dacey R: Spontaneous tone of cerebral parenchymal arterioles: A role in cerebral hyperemic phenomena. J Neurosurg 1989; 71:711–7
30. Kontos HA, Wei EP, Navari RM, Levasseur JE, Rosenblum WI, Patterson JL Jr: Responses of cerebral arteries and arterioles to acute hypotension and hypertension. Am J Physiol 1978; 234:H371–83
31. Kuo L, Davis MJ, Chilian WM: Myogenic activity in isolated subepicardial and subendocardial coronary arterioles. Am J Physiol 1988; 255:H1558–62
32. Nagi M, Ward ME: Modulation of myogenic responsiveness by carbon dioxide in rat diaphragm arterioles: Role of the endothelium. Am J Physiol 1997; 272:H1419–25
33. Toporsian MT, Ward ME: Hyporeactivity of rat diaphragmatic arterioles following exposure to hypoxia in-vivo: Role of the endothelium. Am J Respir Crit Care Med 1997; 156:1572–8
34. Falcone JC, David MJ, Meininger GA: Endothelial independence of myogenic response in isolated skeletal muscle arterioles. Am J Physiol 1991; 260: H130–5
35. Sun D, Messina EJ, Kaley G, Koller A: Characteristics and origin of myogenic response in isolated mesenteric arterioles. Am J Physiol 1992; 263: H1486–91
36. Hwa JJ, Bevan JA: Stretch-dependent (myogenic) tone in rabbit ear resistance arteries. Am J Physiol 1986; 250:H87–95
37. Katusic ZS, Shepherd JT, Vanhoutte PM: Endothelium-dependent contraction to stretch in canine basilar arteries. Am J Physiol 1987; 252:H671–3
38. MacPherson RD, McLeod LJ, Rasiah RL: Myogenic response of isolated pressurized rabbit ear artery is independent of endothelium. Am J Physiol 1991; 260:H779–84
39. Kuo L, Chilian WM, Davis MJ: Interaction of pressure- and flow-induced responses in porcine coronary resistance vessels. Am J Physiol 1991; 261: H1706–15
40. Juncos LA, Garvin J, Carretero OA, Ito S: Flow modulates myogenic responses in isolated microperfused rabbit afferent arterioles via endothelium-derived nitric oxide. J Clin Invest 1994; 95:2741–8
41. Ngai AC, Winn HR: Modulation of cerebral arteriolar diameter by intraluminal flow and pressure. Circ Res 1995; 77:832–40
42. Fujii K, Heistad DD, Faraci FM: Flow-mediated dilation of the basilar artery in vivo. Circ Res 1991; 69:697–705
43. Smiesko V, Kozik J, Dolezel S: Role of endothelium in the control of arterial diameter by blood flow. Blood Vessels 1985; 22:247–51
44. Koller A, Kaley G: Endothelium regulates skeletal muscle microcirculation by a blood flow velocity-sensing mechanism. Am J Physiol 1988; 258:H916–20
45. Gaw AJ, Bevan JA: Flow-induced relaxation of the rabbit middle cerebral artery is composed of both endothelium-dependent and -independent components. Stroke 1993; 24:105–10
46. Bevan JA, Joyce EH, Wellman GC: Flow-dependent dilation in a resistance artery still occurs after endothelium removal. Circ Res 1988; 63:980–5
47. Koller A, Sun D, Huang A, Kaley G: Corelease of nitric oxide and prostaglandins mediates flow-dependent dilation of rat gracilis muscle arterioles. Am J Physiol 1994; 267:H326–32
48. Franchi AM, Chaud M, Rettori V, Suburo A, McCann SM, Gimeno M: Role of nitric oxide in eicosanoid synthesis and uterine motility in estrogen treated rat uteri. Proc Natl Acad Sci U S A 1994; 91:539–43
49. Salvemini D, Misko TP, Masferrer JL, Seibert K, Currie MG, Needleman P: Nitric oxide activates cyclooxygenase enzymes. Proc Natl Acad Sci U S A 1993; 90:7240–4
50. Davidge ST, Baker PN, McLaughlin MK, Roberts JM: Nitric oxide produced by endothelial cells increases production of eicosanoids through activation of prostaglandin H synthase. Circ Res 1995; 77:274–83
51. Hoogerwerf N, van der Linden PJW, Westerhof N, Sipkema P: A new mounting technique for perfusion of isolated small arteries: The effects of flow and oxygen on diameter. Microvasc Res 1992; 44:49–60
52. Hoogerwerf N, Zijlstra EJ, van der Linden PJW, Westerhof N, Siplema P: Endothelium function is protected by albumin and flow-induced constriction is independent of endothelium and tone in isolated rabbit femoral artery. J Vasc Res 1992; 29:367–75
53. Shimoda LA, Norins NA, Madden JA: Flow-induced responses in cat isolated pulmonary arteries. J Appl Physiol 1997; 83:1617–22
54. Garcia-Roldan J-L, Bevan JA: Augmentation of endothelium-independent flow constriction in pial arteries at high intravascular pressures. Hypertension 1991; 17:870–4
55. Henrion D, Laher I, Bevan JA: Intraluminal flow increases vascular tone and Ca2+ influx in the rabbit facial vein. Circ Res 1992; 71:339–45
56. Kuo L, Chilian WM, Davis MJ: Coronary arteriolar myogenic response is independent of endothelium. Circ Res 1990; 66:860–6