Modulation of Rat Pial Arteriolar Responses to Flow by Glucose

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Background: Pial arteriolar responses to flow contribute to regulation of cerebral perfusion and vary according to the transmural pressure to which the vessel is exposed. This study determined the effect of increased glucose concentration on the flow responses of pial arterioles at low and high levels of transmural pressure.

Methods: Pial arterioles from Sprague-Dawley rats were mounted in a perfusion myograph. In some arterioles, the endothelium was removed by perfusion with air. Diameters were recorded at transmural pressures of 60 and 120 mmHg during superfusion with physiologic saline containing 5 mM D-glucose, 20 mM D-glucose, or 5 mM D-glucose and 15 mM L-glucose. Diameters during superfusion with saline containing 44 mM D-glucose were measured at an intraluminal pressure of 60 mmHg. Flow–diameter relationships (5–30 µl/min) were recorded during perfusion with the same solutions.

Results: Increasing D-glucose concentration caused constriction ($P < 0.05$) in endothelium-denuded but not in endothelium-intact arterioles. Addition of L-glucose caused constriction in endothelium-intact and -denuded vessels ($P < 0.05$ for both). At a D-glucose concentration of 5 mM and at low intraluminal pressure, flow elicits endothelium-dependent dilation such that shear stress remains constant. At a D-glucose concentration of 20 or 44 mM, after addition of L-glucose (15 mM), and at high intraluminal pressures, flow elicits constriction and shear stress is unregulated.

Conclusions: High glucose concentrations elicit increased basal arteriolar smooth muscle tone that is counteracted by release of endothelium-derived relaxing factors. Endothelium-dependent relaxation to flow (shear stress) is inhibited at high glucose concentrations.

INTRALUMINAL flow is an important stimulus regulating arteriolar tone in many vascular beds. In arterioles from the pial circulation, increases in flow stimulate endothelium-dependent vasodilation at low transmural pressures.[1–3] As perfusion pressure is increased, however, flow dilation is inhibited, and endothelium-independent constriction becomes the predominant response.[1–3] Because pial vessels play a central role in regulating cerebrovascular resistance,[4] these responses are important determinants of cerebral perfusion and transcapillary fluid flux. The increased incidence of stroke in patients with diabetes mellitus[5,6] and the predisposition to cere-

bral ischemic injury in experimental models of acute hyperglycemia[7] have been attributed to disturbed cerebrovascular hemodynamics. Accordingly, we considered the possibility that high concentrations of glucose may alter the flow responses in pial arterioles in a manner that may contribute to the pathophysiology of abnormal cerebral blood flow regulation in these settings.

The results of previous studies indicate that the effect of increased concentrations of glucose on vascular responses is complex. In isolated cerebral arterial segments, increased glucose enhances basal endothelial vasodilator release.[8] The response to pharmacologic agents that elicit dilation by stimulating release of these same mediators, however, is reduced,[9–12] raising the possibility that other endothelium-dependent responses, such as flow dilation, may also be impaired. In vascular smooth muscle, increasing glucose concentration was found to increase intracellular calcium levels[13,14] and to activate protein kinase C,[15] which would enhance basal tone. Others, however, have reported that elevated glucose inhibits voltage-activated Ca^{2+} entry and receptor-mediated Ca^{2+} release,[16,17] suggesting that contractile responses to mechanical stimuli may be similarly affected. Accordingly, we proposed that acute increases in glucose concentration will alter the intrinsic responses of pial arterioles through effects on endothelial and smooth muscle function and that the effect on basal tone may differ from that on their reactivity to mechanical stimuli. Specifically, we predicted that increased glucose will enhance smooth muscle tone at a given intraluminal pressure, increase basal endothelium-derived relaxing factor release, and inhibit endothelium-dependent and endothelium-independent responses to flow.

Methods

The experimental protocol was approved by the animal care committee of the Montreal Neurologic Institute, Montreal, Quebec, Canada. Male Sprague-Dawley rats (weight, 200–250 g) were killed by decapitation. The entire brain was immediately removed and placed in a silicone-lined dissecting dish containing oxygenated physiologic salt solution (PSS; in mM, NaCl, 119; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$, 1.17; $NaHCO_3$, 24.9; EDTA, 0.4; $CaCl_2$, 3.7; glucose, 5; pH, 7.4) at 4°C. An unbranched arteriolar segment at least 1 mm in length from the territory of the posterior cerebellar artery was dissected free, cleared from the adhering tissue, and transferred to a plexiglas vessel chamber (Living Systems, Burlington, VT) containing PSS and mounted on inflow and outflow

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micropipettes matched for resistance to flow. The system was arranged to have mirror symmetry, with the axis of symmetry located at the middle of the segment. The inflow cannula was connected to a calibrated constant flow pump (Living Systems) taking its inflow from a reservoir of PSS. The outflow cannula was connected to a pump that was regulated by a servo system to maintain a constant downstream pressure. This allowed the vessel to be perfused at constant flow while the midpoint pressure (calculated as the average of the upstream and downstream pressures[3,18]) was maintained at the desired level by adjusting the downstream pressure target. 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. 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 segment were made using a high resolution CCD video camera (Hitachi KPC503, Hitachi, San Jose, CA) and a video caliper (Living Systems) calibrated using a stage micrometer. The vessel was superfused with PSS at a rate of 6 ml/min, and the chamber was maintained at 37°C. 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 (AVL Instruments, model 995, Graz, Austria). A plexiglas cover excluded ambient air from the chamber. The reservoir containing the perfusate and superfusate and the vessel chamber itself were bubbled with gas whose composition was adjusted, using separate tanks and regulators for each of oxygen, carbon dioxide, and nitrogen, to achieve P_{O_2} and P_{CO_2} 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. After 60 min of equilibration, steady state diameters were recorded, and endothelium-dependent and -independent dilation were evaluated in all vessels by determining the ability of acetylcholine (Ach, 10^{-5} M, Sigma, St. Louis, MO) and the NO donor diethylaminenitric oxide (DEA/NO, 10^{-4} M, Research Biochemicals International, Natick, MA), respectively, to inhibit intrinsic tone. In vessels from which endothelium was to be removed, the intraluminal pressure was 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 min at 20 mmHg. The outflow cannula was closed, the intraluminal pressure was restored to 60 mmHg, and the dila-

tory responses to Ach and DEA/NO were once again determined. Elimination of vasodilatation in response to Ach with an intact vasodilator response to DEA/NO after this procedure was taken as evidence of functional endothelial ablation.[3,19]

Because the response to flow varies with the level of basal tone,[1–3] the effects of increasing glucose concentration were studied at an intraluminal pressure of 60 mmHg, which is within the physiologic range for rat arterioles of this size,[3] and at 120 mmHg, a point near the upper end of the myogenically regulated pressure range.[3] The specificity of the effect of glucose was tested using the metabolically inactive stereoisomer L-glucose. After the equilibration period, the distending pressure was either maintained at 60 mmHg or slowly increased to 120 mmHg. Endothelium-intact and -denuded arterioles from each of these groups were superfused with PSS containing 5 mM D-glucose, 20 mM D-glucose, or 5 mM D-glucose and 15 mM L-glucose (n = 6 per condition). In 12 vessels (6 with endothelium intact and 6 with endothelium removed) maintained at an intraluminal pressure of 60 mmHg, the superfusate and perfusate solutions were replaced with PSS containing 44 mM D-glucose. Vessels were allowed to equilibrate a further 30 min at the final solute concentration, and flow responses were evaluated by perfusing the vessels with PSS identical in composition to the superfusate. Flow was increased from 0 to 30 μl/min in 5-μl/min steps while maintaining the intraluminal pressure at the baseline value (either 60 mmHg or 120 mmHg). Diameter was recorded after the vessel had reached steady state, 10 min after each flow step.

In separate groups of endothelium-intact arterioles, after stabilization at 60 mmHg or 120 mmHg, the responses to Ach and DEA/NO were evaluated as previously described. Either N^G-nitro-L-arginine methylester (L-NAME, 10^{-5} M) or indomethacin (10^{-5} M) was then added to the superfusate of six vessels in each group. In a previous study,[3] this concentration of L-NAME was found to eliminate dilation to Ach without affecting the response to prostaglandin E_2 (PGE_2) or DEA/NO in rat pial arterioles. Indomethacin in this concentration has been shown to block dilation to arachadonic acid in rat arterioles comparable in diameter with those currently under study[20] and does not alter the responses to Ach, DEA/NO, or PGE_2 in rat pial arterioles.[3] After 15 min of exposure to these agents, the superfusate was changed to PSS containing 20 mM D-glucose and either L-NAME or indomethacin, respectively. The flow–diameter relationships were then determined at a distending pressure of 60 or 120 mmHg.

The effects of changing glucose concentration and osmolarity on the vasodilator response to Ach and DEA/NO were evaluated in separate groups of endothelium-intact arterioles (n = 6 per group). After equilibration, responses to Ach and DEA/NO were tested at an

intraluminal pressure of 60 mmHg during superfusion with PSS containing 5 mM D-glucose as above and confirmed that endothelium-dependent and -independent responses were intact. The distending pressure was either maintained at 60 mmHg or slowly increased to 120 mmHg, and the vessels were equilibrated a further 30 min, during which they were superfused with PSS containing 5 mM D-glucose, 20 mM D-glucose, 44 mM D-glucose, or 5 mM D-glucose and 15 mM L-glucose. After recording the baseline diameters, the steady-state diameters achieved after the addition of Ach (10^{-5} mM) to the superfusate were recorded. Ach was then removed from the superfusate, and the vessels were allowed to return to their baseline diameters. DEA/NO (10^{-4} mM) was then added to the superfusate, and the steady state diameters were recorded.

Diameter is normally regulated to preserve shear stress at the endothelial–luminal interface within a narrow range, suggesting that shear, not flow, is the stimulus for endothelial mediator release.[21-23] Because of their effects on viscosity, changes in solute concentration will alter shear and, therefore, the response to flow. To control for this effect, diameter was also plotted against shear stress (τ), calculated at the steady-state diameter that followed each flow step as $\tau = 4\eta Q/\pi r^3$, where η is viscosity (Poises), Q is flow (ml/s), and r is vessel radius (cm). Viscosities, measured against water in a viscometer (Cannon Instrument Co., State College, PA) at 37°C, were 0.00665, 0.007195, 0.00755, and 0.007225 Poises for the 5 mM D-glucose, 20 mM D-glucose, 44 mM D-glucose, and 5 mM D-glucose and 15 mM L-glucose PSS solutions, respectively.

Statistical Analysis

Differences among multiple means were detected by analysis of variance (ANOVA) 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 (n = number of animals) with $P < 0.05$ representing statistical significance.

Results

The internal diameters for all vessels studied averaged 118 ± 12 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 (161 ± 11 μm; $P < 0.05$ for difference), indicating that by the end of the equilibration period the arterioles had developed spontaneous tone. The average diameters during initial relaxation with Ach (136 ± 10 μm) and DEA/NO (159 ± 13 μm) were also significantly greater ($P < 0.05$ for both) than at the end of the equilibration period. The diameter during treatment with DEA/NO did not differ from that recorded during passive conditions.
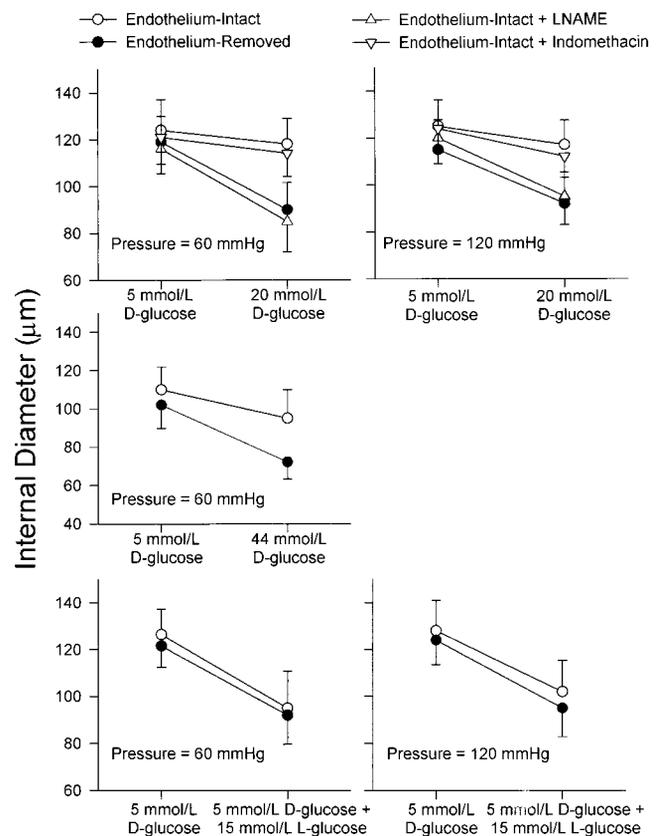

Fig. 1. (*Top*) Effect of increasing superfusate D-glucose concentration from 5 mM to 20 mM on diameter of endothelium-intact and -denuded arterioles at intraluminal pressures of 60 and 120 mmHg and of endothelium-intact arterioles pretreated with N^G-nitro-L-arginine methylester (L-NAME, 10^{-5} M) and indomethacin (10^{-5} M) at 60 mmHg. $P < 0.05$ for difference between endothelium-intact and -denuded arterioles and for difference between endothelium-intact arterioles with and without L-NAME. (*Middle*) Effect of increasing superfusate D-glucose concentration to 44 mM on diameter of endothelium-intact and -denuded arterioles at an intraluminal pressure of 60 mmHg. $P < 0.05$ for difference between endothelium-intact and endothelium-denuded groups. (*Bottom*) Effect of changing superfusate solution from physiologic saline containing 5 mM D-glucose to 5 mM D-glucose plus 15 mM L-glucose in arterioles with and without endothelium at intraluminal pressures of 60 mmHg and 120 mmHg.

The effects of changing the superfusion solution from PSS containing 5 mM D-glucose to PSS containing 20 mM D-glucose on the steady state diameter of endothelium-intact and -denuded arterioles at transmural pressures of 60 mmHg and 120 mmHg in the absence of luminal flow, and the effects of L-NAME and indomethacin on these effects in endothelium-intact arterioles are illustrated in the top panel of figure 1. The middle panel of figure 1 presents the response to changing the superfusion solution to PSS containing 44 mM D-glucose in arterioles pressurized to 60 mmHg. The effects of changing the superfusion solution from PSS containing 5 mM D-glucose to PSS containing 5 mM D-glucose and 15 mM L-glucose on the diameters of endothelium-intact and -de-

nuded arterioles at distending pressures of 60 and 120 mmHg are presented in the bottom panel of figure 1.

In endothelium-intact arterioles, changing the superfusion solution to PSS containing 5 mM D-glucose and 15 mM L-glucose elicited constriction, whereas the change in diameter during superfusion with PSS containing 20 mM or 44 mM D-glucose did not reach statistical significance. Arterioles from which the endothelium had been removed constricted after changing the superfusion solution to PSS containing 20 mM D-glucose, 44 mM D-glucose, or 5 mM D-glucose and 15 mM L-glucose. There was no difference between endothelium-intact and endothelium-removed groups exposed to PSS containing 5 mM D-glucose and 15 mM L-glucose. In endothelium-intact arterioles, pretreatment with L-NAME had the same effect on the response to increasing the glucose concentration to 20 mM as removing the endothelium, whereas indomethacin had no effect.

The relationships between flow and diameter and between shear stress and diameter in endothelium-intact and -denuded arterioles pressurized to 60 mmHg, perfused and superfused with PSS containing 5 mM, 20 mM, or 44 mM D-glucose are compared in figure 2. The effects of L-NAME and indomethacin on these relationships in endothelium-intact arterioles during perfusion and superfusion with PSS containing 20 mM D-glucose at 60 mmHg distending pressure are illustrated in the top panel. In endothelium-intact vessels, perfused and superfused with PSS containing 5 mM D-glucose, increases in flow elicited dilation such that shear stress remained constant over a range of flows. In contrast, vessels exposed to 20 mM and 44 mM D-glucose did not dilate in response to increases in flow, and shear stress was unregulated. In endothelium-denuded arterioles pressurized to 60 mmHg, increasing flow and shear stress resulted in constriction, with vessels exposed to 20 mM and 44 mM D-glucose reaching smaller diameters and higher shear stress levels than those superfused and perfused with PSS containing 5 mM D-glucose. In endothelium-intact arterioles exposed to 20 mM D-glucose and treated with L-NAME, flow–diameter and shear stress–diameter relationships did not differ from those in untreated vessels from which the endothelium had been removed. Indomethacin did not alter the flow response.

Figure 3 presents the flow–diameter and shear stress–diameter relationships in endothelium-intact and -denuded arterioles perfused and superfused with PSS containing 5 mM and 20 mM D-glucose at an intraluminal pressure of 120 mmHg. At both glucose concentrations, increasing flow and shear stress elicited constriction. In endothelium-denuded, but not endothelium-intact, arterioles, diameters were significantly smaller during superfusion and perfusion with PSS containing 20 mM D-glucose than in those exposed to 5 mM D-glucose.

In figure 4, the relationships between flow and diameter and between shear stress and diameter in endothe-
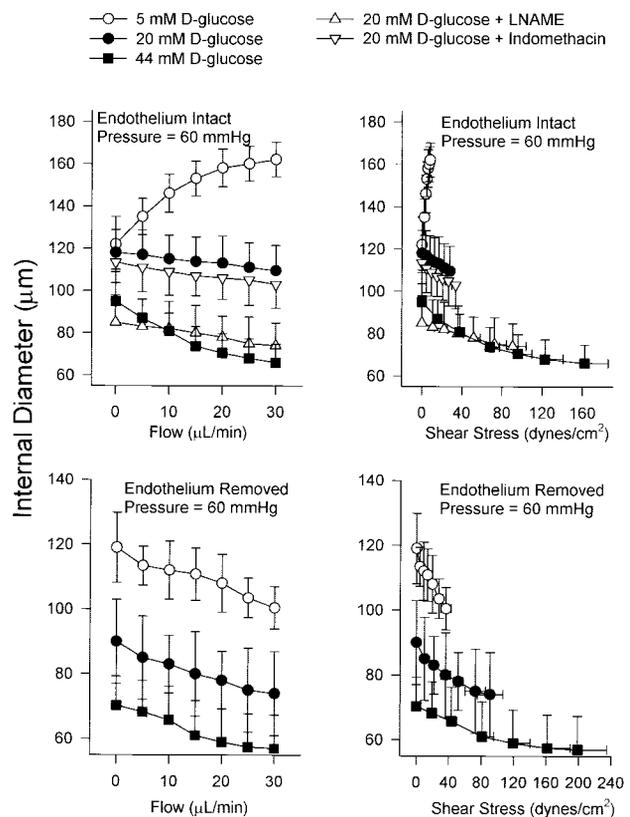

Fig. 2. Relationships between flow and diameter and between shear stress and diameter in endothelium-intact and -denuded arterioles pressurized to 60 mmHg during perfusion and superfusion with physiologic saline containing 5 mM, 20 mM, or 44 mM D-glucose and the effects of N^{G}-nitro-L-arginine methylester (L-NAME) and indomethacin on these relationships in endothelium-intact arterioles exposed to 20 mM D-glucose. $P < 0.05$ for differences between 5 mM and 20 mM D-glucose and between 5 mM and 44 mM D-glucose in endothelium-intact and -denuded vessels. $P < 0.05$ for difference between L-NAME–treated and –untreated endothelium-intact arterioles exposed to 20 mM D-glucose.

lium-intact and -denuded arterioles perfused and superfused with PSS containing 5 mM D-glucose and 15 mM L-glucose at intraluminal pressures of 60 and 120 mmHg are illustrated. Increases in flow and shear stress elicited constriction in all arterioles. There was no difference between endothelium-intact and -removed groups at either intraluminal pressure level. The flow–diameter and shear stress–diameter relationships in these vessels did not differ from those recorded in endothelium-denuded arterioles exposed to 20 mM D-glucose.

Table 1 presents the effect of Ach and DEA/NO on steady state diameters of endothelium-intact arterioles during superfusion with PSS containing 5 mM and 20 mM D-glucose or 5 mM D-glucose and 15 mM L-glucose at intraluminal pressures of 60 and 120 mmHg and of arterioles pressurized to 60 mmHg during superfusion with PSS containing 44 mM D-glucose. Changes in extracellular glucose concentration and osmolarity had no effect on maximal relaxation to DEA/NO; however, conditions
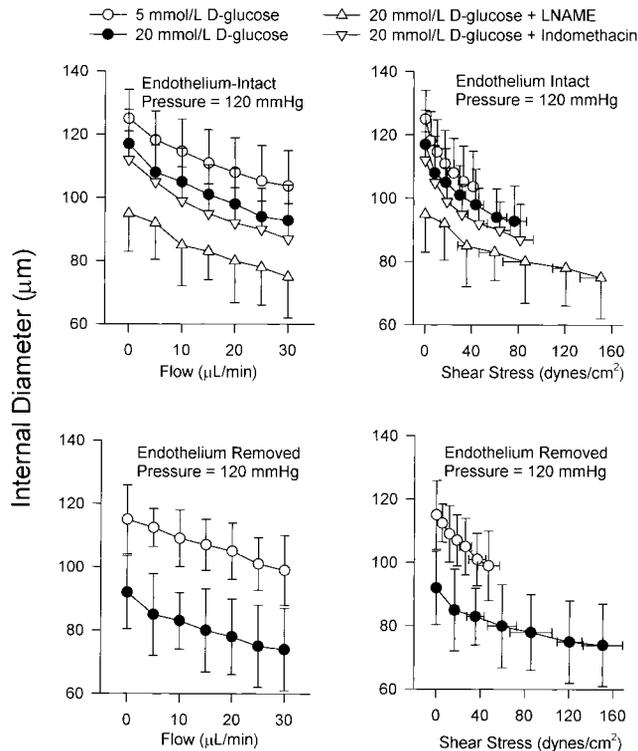
Fig. 3. Relationships between flow and diameter and between shear stress and diameter in endothelium-intact and -denuded arterioles pressurized to 120 mmHg during perfusion and superfusion with physiologic saline containing 5 mM D-glucose or 20 mM D-glucose. $P < 0.05$ for difference between endothelium-denuded arterioles exposed to 5 mM D-glucose and those exposed to 20 mM D-glucose.

that inhibited the flow response also decreased the responses to Ach.

Discussion

The results of this study indicate that (1) in pial arterioles from which the endothelium has been removed, increasing D-glucose concentration or osmolarity (by adding L-glucose) in the superfusion buffer causes constriction; (2) in endothelium-intact arterioles, increasing the concentration of D-glucose, but not adding L-glucose, activates endothelium-dependent mechanisms that inhibit this increase in tone, an effect that is eliminated by L-NAME but not affected by indomethacin; (3) at a D-glucose concentration of 5 mM, in endothelium-intact arterioles pressurized to 60 mmHg, increasing flow elicits dilation such that shear stress changes little despite large variations in flow; and (4) increasing glucose concentration or osmolarity inhibits endothelium-dependent flow dilation but not endothelium-independent flow constriction.

Alterations in cerebral blood flow have been demonstrated in patients with diabetes mellitus,[5,6,24] and elevated basal cerebral blood flow has been documented in some animal models of experimentally induced diabe-
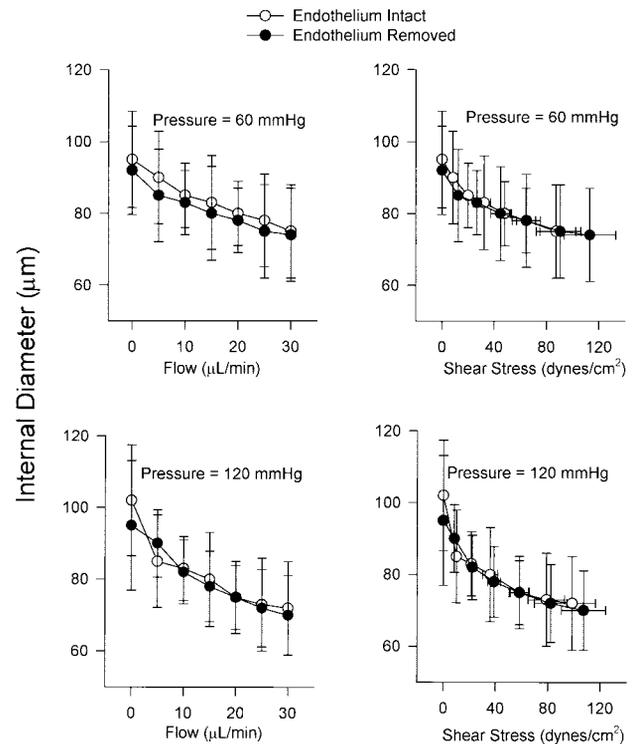

Fig. 4. Relationships between flow and diameter and between shear stress and diameter in endothelium-intact and -denuded arterioles pressurized to 60 mmHg and 120 mmHg during perfusion and superfusion with physiologic saline containing 5 mM D-glucose plus 15 mM L-glucose.

tes.[25,26] The effect of acute increases in blood glucose *in vivo* on cerebral blood flow has been more variable. In nondiabetic rats, acute hyperglycemia (39.3 ± 2.9 mM) caused a small decrease in cerebral blood flow.[27] In contrast, Sieber *et al.*[28] found, in nondiabetic dogs, that acute hyperglycemia (14.94 ± 2.52 mM) was associated with increased cerebral blood flow and decreased cerebrovascular resistance. The difference between these results has been attributed to difficulties in isolating direct effects of glucose concentration from compensatory responses to changes of cardiac output and transmural pressure fluctuations resulting from effects on vascular tone upstream of the vessels regulating cerebral blood flow.[8,28]

Our current results in isolated endothelium-denuded pial vessels indicate that increasing D-glucose concentration enhances basal pressure-sensitive tone. This is a nonspecific effect of increasing osmolarity because the same response was elicited by exposing the vessels to the metabolically inert isomer L-glucose. Consistent with this finding, increased osmolarity has been reported to enhance basal tone in arterial strips *in vitro*.[29,30] In segments of cerebral arteries, larger than those used in the current study, however, others failed to demonstrate any effect of L-glucose on basal tone,[8] and hyperosmolar solutions have been found to decrease agonist-induced contraction through effects on smooth muscle cell mem-

Table 1. Endothelium-dependent and Independent Dilation

	Pressure (mmHg)	Baseline (μm)	Ach (μm)	DEA/NO (μm)
5 mM D-glucose	60	116 ± 13	156 ± 10*	178 ± 13*
	120	125 ± 9	132 ± 10	188 ± 18*
20 mM D-glucose	60	118 ± 11	124 ± 13	175 ± 17*
	120	117 ± 11	125 ± 12	192 ± 15*
44 mM D-glucose	60	104 ± 15	110 ± 10	175 ± 13*
5 mM D-glucose + 15 mM L-glucose	60	96 ± 13	98 ± 10	182 ± 14*
	120	102 ± 15	104 ± 13	190 ± 12*

* $P < 0.05$ for change from baseline.

brane ion conductance in rabbit ear artery.[31] Because, in most arterial beds, pressure-sensitive tone is enhanced in smaller compared with larger vessels,[32] it may be anticipated that changes in this parameter in response to changes in the composition of the extracellular milieu may demonstrate similar size dependency and result in the apparent discrepancy between our present results and those reported previously in larger vessels. The prevailing level of myogenic tone is a primary determinant of the direction of the flow response in the cerebral vasculature.[1–3,33] Because these vessels constrict or dilate in response to flow at high and low levels of basal tone, respectively, enhanced pressure sensitivity of pial arteriolar tone, unopposed by the concomitant inhibitory effect exerted by the endothelium, could explain our finding that the normal flow response at low perfusion pressures is reversed in the presence of hyperosmolar solutions.

Cipolla *et al.*[8] have reported that increasing glucose in the superfusing buffer elicits endothelium-dependent vasodilation in isolated cerebral arterial segments and that this effect is blocked by L-arginine analogs. Although, in the current study, the net result of endothelial mediator release in response to increased glucose concentration was maintenance of the baseline diameter rather than dilation, the effect was sufficient to completely oppose the vasoconstriction observed in arterioles from which the endothelium had been removed. Treatment with L-NAME reproduced the effect of endothelial ablation, supporting the assertion[8] that the response requires nitric oxide synthesis. As in the study of Cipolla *et al.*,[8] indomethacin had no effect on the steady-state diameter during exposure to increased glucose concentration, arguing against a role for altered release of vasoconstrictor[34] or vasodilator prostaglandins.[35] Because the inhibitory effect on tone was not seen in vessels in which L-glucose had been added to the superfusate, we infer that it requires glucose to be available as a metabolic substrate.

Studies in isolated arterial preparations and *in vivo* measurements[9–12,36–39] have indicated that vasodilatory responses to agonists that stimulate endothelial nitric oxide release are decreased during hyperglycemia in the cerebral circulation. Taken together, therefore, the available data suggest that glucose exerts a dual effect, acting as a stimulus for basal endothelium-derived relaxing fac-

tor release while inhibiting the capacity for stimulation of their production. Acute hyperglycemia induced by oral glucose loading has been shown to impair brachial arterial flow-mediated dilation (after arterial occlusion and release) in nondiabetic adult humans.[40] The effect of increased glucose concentration on the change in perfusion pressure during increases in coronary artery flow has also been studied in the isolated pump-perfused beating guinea pig heart.[41] In that study, increasing D-glucose concentration in the perfusing buffer to 44 mM, in the presence of indomethacin, enhanced the reduction in resistance that accompanied increases in flow, and an equimolar concentration of D-mannitol did not reproduce this effect. Our current results, in pial arterioles, demonstrate that endothelium-dependent flow relaxation is impaired by acute exposure to high glucose concentrations and, therefore, are compatible with previous observations in human brachial artery but oppose those reported in the guinea pig coronary circulation. The inhibition of flow dilation in the present study cannot be a reflection of osmolarity-induced increase in basal tone because baseline diameter was not affected by the change in glucose concentration in endothelium-intact vessels.

In previous studies, the addition of L-glucose or D-mannitol, as hyperosmotic controls, did not have the same effect on the response to pharmacologic agonists of endothelium-dependent vasorelaxation[9–12] or on flow dilation[41] as did increasing D-glucose concentration. In contrast, in the present study, L-glucose eliminated flow dilation as effectively as did increasing the concentration of the D-isomer. This does not, however, imply that the inhibitory effects of the D- and L-isomers on flow dilation are mediated by the same mechanism in these vessels. In contrast to the effect of increasing D-glucose concentration, the addition of L-glucose to the superfusate elicited significant vasoconstriction in endothelium-intact and -denuded arterioles (fig. 1). This increase in basal smooth muscle tone may, therefore, be sufficient to inhibit flow dilation[1–3,33] independent of any osmolarity-induced effect on endothelial function.

The current results do not support our original hypothesis that increased glucose concentration will inhibit endothelium-independent flow constriction in these arterioles. In endothelium-denuded arterioles, diameters

were smaller and the levels of shear stress achieved were higher during perfusion and superfusion with 20 mM and 44 mM than with 5 mM D-glucose. The mechanisms that mediate flow constriction in cerebral arterioles are unknown. In the rabbit facial vein, the response is dependent on extracellular sodium concentration,[42] and in cat pulmonary arterioles, it requires participation of intracellular Ca^{2+} stores and activation of protein kinase C.[43] Although some of these mechanisms also participate in receptor-mediated contraction, the reported susceptibility of agonist activation of vascular smooth muscle to inhibition by glucose[15-17] suggests that other pathways are involved in those responses that are more sensitive to the effects of glucose concentration than is flow constriction.

To the extent that our findings in isolated arterioles may apply in the intact pial circulation, they predict the loss of flow dilation in conditions associated with acute hyperglycemia. Flow dilation is required to maximize tissue perfusion,[44,45] and an important role in maintaining tissue blood flow in proportion to metabolic demand has been proposed.[3,46,47] The possibility that inhibition of this response may contribute to the pathophysiology of cerebral ischemic injury in conditions associated with acute hyperglycemia, therefore, merits investigation.

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