Role of Endothelium in Shear Stress–Induced Constrictions in Rat Middle Cerebral Artery

Robert M. Bryan, Jr, PhD; Marie L. Steenberg, MS; Sean P. Marrelli, PhD

Background and Purpose—Luminal shear stress has been reported to constrict cerebral arteries and arterioles of several species. Although the endothelium is not required for this response, it is not known whether the endothelium enhances or attenuates shear stress–induced constrictions.

Methods—Middle cerebral arteries (MCAs) were isolated from male Long-Evans rats, mounted in a tissue bath, and pressurized to 80 mm Hg in the absence of luminal flow. In some MCAs, the endothelium was selectively loaded with fura 2 for the measurement of endothelial Ca^{2+} concentration. Luminal shear stress was increased by adjusting luminal flow while maintaining a constant intraluminal pressure.

Results—After the development of spontaneous tone in MCAs without luminal flow, inside diameters were ≈190 μm. MCAs constricted ≈15% when luminal flow was increased to produce a shear stress of 50 dyne/cm^2. The shear stress–induced constrictions were more pronounced in vessels without intact endothelium. Scavenging reactive oxygen species with 4,5-dihydroxy-1,3-benzene disulfonic acid (Tiron) or superoxide dismutase/catalase significantly inhibited the shear stress–induced constrictions in vessels with intact endothelium and in vessels in which the endothelium had been removed. In intact vessels, endothelial Ca^{2+} increased 33 nmol/L (from 133±11 to 166±12 nmol/L) when shear stress was increased to 50 dyne/cm^2. The presence of N^G-nitro-L-arginine methyl ester (L-NAME), L-NAME+indomethacin, or L-NAME+indomethacin+charybdotoxin had no significant effect on the shear stress–induced constrictions in MCAs with intact endothelium.

Conclusions—We conclude that the endothelium plays a role in attenuating the shear stress–induced constrictions in rat MCAs. The attenuation does not appear to be by release of NO, prostacyclin, or endothelium-derived hyperpolarizing factor. The endothelium apparently attenuates the constriction by an unknown dilating factor, by a dilating process, or simply by attenuating the mechanical force of the shear stress as it is transmitted to the abluminal side of the vessel. **(*Stroke*. 2001;32:1394-1400.)**

Key Words: calcium ■ endothelium ■ fura 2 ■ middle cerebral artery ■ reactive oxygen species ■ rats

Peripheral arteries and arterioles dilate when flow through the lumen of the vessel is increased. The mechanism involves sensing a mechanical deformation (resulting from shear stress) by the endothelium and releasing a relaxing factor (NO or prostacyclin).[1–4] In cerebral vessels, the response to shear stress is somewhat more controversial. Shear stress has been reported to produce dilations, constrictions, or a combination of dilation and constriction depending on the level of shear stress (see Bryan et al[5] for complete references). The controversy surrounding the effect in cerebral vessels could possibly be dependent on species, vessel size, or vessel location in the vascular tree.

In middle cerebral arteries (MCAs) from the cat and rat and penetrating arterioles from the rat, luminal flow constricted the vessels in a shear stress–dependent manner.[5,6] Additionally, flow constricted rabbit cerebral vessels during some but not all conditions.[7,8] The shear stress–dependent constriction persisted even after removal of the endothelium. The role of the endothelium is complicated in that it is not needed for a shear stress–induced constriction to occur, but endothelia are the cells that directly experience deformation by shear stress.[5,6] Neither NO nor cyclooxygenase metabolites (ie, prostacyclin) appeared to be involved with the response in endothelium-intact cat MCAs. The exact role of the endothelium in the shear stress–induced constrictions remains largely unknown, especially when rat cerebral vessels are considered. Consequently, it is possible that the endothelium attenuated the constriction, enhanced the constriction, or had no effect on the response. Attenuation of the shear stress–induced constriction could be a result of the release of NO, prostacyclin, endothelium-derived hyperpolarizing factor (EDHF), or reactive oxygen species (ROS). Enhancement of the shear stress–induced constriction could be a result of constricting factors such as ROS. The rationale for considering ROS is

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that endothelial cells in culture generate ROS when exposed to shear stress.[9–11] Furthermore, ROS have been reported to either dilate or constrict cerebral vessels.[6,12,13]

The purpose of the present study was to determine the role of endothelium in the shear stress–induced constriction in the rat middle cerebral artery. Specifically, we tested the following hypotheses: (1) ROS that are produced by the endothelium as a result of shear stress either attenuate or enhance the shear stress–induced constriction. (2) Endothelium attenuates the shear stress–induced constrictions in rat MCAs by releasing NO, prostacyclin, or EDHF.

Materials and Methods

Harvesting and Mounting Vessels

The Animal Protocol Review Committee at Baylor College of Medicine approved the experimental protocol. Male Long-Evans rats (250 to 350 g) were anesthetized with 3% isoflurane and decapitated. The brain was immediately removed and placed in cold (4°C) physiological saline solution (PSS). MCAs were carefully harvested and mounted in a vessel chamber maintained at 37°C.[14] Micropipettes were inserted into both ends of each vessel, and the vessel was secured with nylon ties. Luminal pressure was set at 80 mm Hg by raising 2 reservoirs that were connected to the micropipettes.[14] The vessels were bathed in PSS that was equilibrated with a gas consisting of 20% O_2/5% CO_2, with a balance of N_2. The pH of the bath was ≈7.40, P_{CO_2} was ≈35 mm Hg, and P_{O_2} was ≈130 mm Hg.[14]

Flow through the lumen of the vessels was produced by a variable-speed syringe pump (model 22, Harvard Apparatus) (see Bryan et al[5] for diagram and more details). Pressure transducers on either side of the vessel chamber provided a measurement of perfusion pressure. Before mounting of the vessel, the resistance of the tubing and micropipettes on either side of the vessel was measured. From the resistances of the micropipettes, an algorithm was used to determine the upstream pressure and downstream pressure necessary to maintain a luminal pressure of 80 mm Hg.[5] After initiating or increasing luminal flow, the output reservoir was appropriately lowered as calculated by the algorithm.

The vessels were magnified with an inverted microscope equipped with a video camera and monitor. Inside diameters of the vessels were measured manually from the video screen or from videotape made at the time of the study. After they were mounted and pressurized, the vessels of all groups developed spontaneous tone (≈18% to 20%). Experimental protocols were not initiated until the vessel diameters were stable over a period of 15 minutes.

Shear stress was calculated by use of the following equation[1,5,15,16]: $t=4\eta Q/\pi r^3$, where t is shear stress in dyne/cm^2, η is viscosity, Q is flow, and r is the inside radius of the vessel. In all studies, we attempted to set a flow to produce a given shear stress. Because the diameter changed when flow was altered, adjustments in flow were made 3 times to approach the target shear stress.

The presence of intact endothelium in cerebral vessels was verified by luminal administration of ATP, an agonist for $P2Y_2$ receptors. ATP dilates cerebral arteries and arterioles via an endothelium-dependent mechanism involving NO and EDHF.[17–19] In some arteries, the endothelium was damaged by passing air through the lumen of the vessel as previously described.[14,17,18] The absence of dilation to luminally applied ATP indicated that the endothelium had been successfully removed. Vessels denuded of endothelium dilated in response to the NO donor, *S*-nitroso-*N*-acetylpenicillamine (SNAP), indicating that the vascular smooth muscle was intact. In studies in which the endothelium was damaged or in which NO synthase was inhibited with N^G-nitro-L-arginine methyl ester (L-NAME), the vessels constricted ≈20% of the resting diameter.[19] SNAP was added to these vessels to dilate them back to the diameter before L-NAME treatment or damage to the endothelium. In another group of MCAs, endothelial cell rigidity was increased by exposing the luminal surface to glutaraldehyde (0.025%) for 20 seconds. This procedure has been reported to increase the shear modulus of the endothelium by >10-fold.[20]

Measurement of Endothelial Ca^{2+} With Use of Fura 2

Ca^{2+} concentrations in the cytoplasm of the endothelium and vessel diameter were simultaneously measured as previously described.[21] Briefly, fura 2-AM (0.67 μmol/L final concentration) was added to the luminal perfusate. After 5 minutes of exposure, the vessel was washed to remove extracellular fura 2-AM, and an additional 30 minutes for was allowed for intracellular deesterification of the fura 2-AM to fura 2. The addition of fura 2-AM through the lumen at the above concentration and duration selectively loads the endothelium.[21] Therefore, the fluorescence signal is derived exclusively from the endothelium, and the calculated Ca^{2+} concentration represents only the endothelium.[21] For Ca^{2+} measurements, the vessels were illuminated with excitation light alternating between wavelengths of 340 and 380 nm with the use of a xenon arc lamp, appropriate filters, and a filter changer (Intracellular Imaging). Additionally, red light from a separate lamp was used to transilluminate the vessels for diameter measurements. The light was collected with a quartz objective (×10, numerical aperture 0.5, Nikon) and subsequently split and filtered with a dichroic mirror. The red light was diverted to a charge-coupled device for diameter measurements, and the remainder was diverted to a photomultiplier after passing through a 510-nm narrow bandpass filter. Intensities of the 510-nm fluorescence light were used to quantify intracellular Ca^{2+} according to the following equation: $[Ca^{2+}]_i=\beta(R-R_{min})K_d/(R_{max}-R)$, where $[Ca^{2+}]_i$ is the intracellular Ca^{2+} concentration in the endothelium, β is the ratio of the 380-nm fluorescence intensity for Ca^{2+}-unbound fura 2 over Ca^{2+}-bound fura 2, R is the ratio of light intensity at 510 nm when excited at 340 nm to the intensity when excited at 380 nm (340/380 ratio) at a given condition (ie, shear stress), R_{min} is the 340/380 ratio at zero $[Ca^{2+}]_i$, R_{max} is the 340/380 ratio when $[Ca^{2+}]_i$ was sufficiently high to saturate fura 2, and K_d is 282 nmol/L. β, R_{min}, and R_{max} were determined in a separate group of vessels as previously described.[21]

Drugs and Reagents

ATP, 2-methylthio-ATP (MeSATP), indomethacin, 4,5-dihydroxy-1,3-benzene disulfonic acid (Tiron), charybdotoxin, and L-NAME were purchased from Sigma Chemical Co. SNAP was purchased from Research Biochemicals Inc. Catalase was purchased from ICN Biochemicals. Superoxide dismutase (SOD) was purchased from Calbiochem). Fura 2-AM (50 μg) was purchased from TefLabs and dissolved in 75 μL dimethyl sulfoxide (containing 14% pluronic solution).

PSS consisted of the following (mmol/L)[14]: NaCl 119, $NaHCO_3$ 24, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $CaCl_2$ 1.6, glucose 5.5, and EDTA 0.026.

Statistical Analysis

All data are presented as mean±SE. For statistical analysis, the 1- or 2-way repeated-measures ANOVA was used with a post hoc Tukey test (where appropriate) for comparison of individual groups and individual data points. The acceptable level of significance was defined as $P<0.05$.

Results

Figure 1A shows absolute changes in diameter of the rat MCAs when luminal shear stress was increased in control MCAs and in MCAs treated with a scavenger of ROS, Tiron (10 mmol/L) or SOD (200 U/mL)/catalase (140 U/mL) (SOD/cat). Note that for each group, the MCAs were allowed to develop spontaneous tone in the absence of luminal flow. The SOD/cat group had a mean inner diameter that was greater than that in the control or Tiron group; however, there was no group difference ($P=0.11$, 2-way repeated-measures ANOVA). Furthermore, because the MCAs were randomized

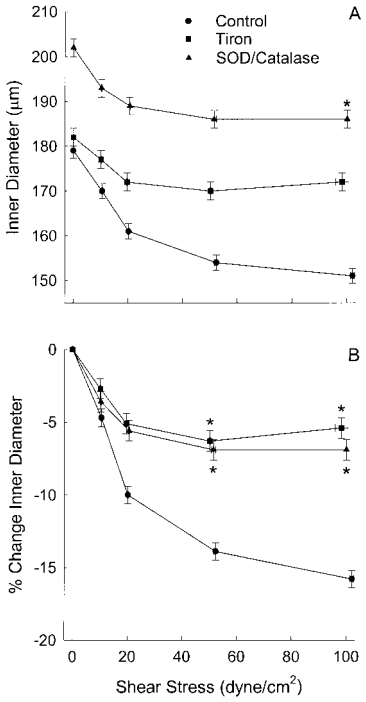

Figure 1. A, Effect of the scavengers of ROS, Tiron (10 mmol/L) or a combination of SOD (200 U/mL)/catalase (140 U/mL), on the shear stress–induced constriction of the rat MCA. There was no significant group difference ($P=0.11$), but there was a significant shear stress effect ($P<0.001$) and significant interaction between the treatment groups and shear stress ($P<0.001$, 2-way repeated-measures ANOVA). The number of observations was 8 for the control group and 6 each for the Tiron and SOD/catalase groups. *$P<0.05$ compared with the diameter of the control group at 100 dyne/cm^2 (Tukey test). B, Same data as shown in panel A, except that the diameters were plotted as a percent change from the resting diameter (no shear stress, or 0 dyne/cm^2). There was a significant group effect ($P=0.01$), a significant shear stress effect ($P<0.001$), and a significant interaction between groups and shear stress ($P<0.001$). The control group was significantly different from the Tiron group ($P=0.02$) and the SOD/catalase group ($P=0.04$, Tukey test). *$P<0.05$ compared with the corresponding diameter of the control group (Tukey test).

and because there was no significant effect of SOD/cat on the vessel diameter, this increased mean diameter was due to chance alone. The magnitude of constriction due to shear stress for the Tiron and SOD/cat groups was less than that for the control group. This can be clearly seen in Figure 1B, where the same data were plotted as percent change in inner diameter. For the results in Figure 1B, there was a significant group effect ($P=0.01$), a significant shear stress effect ($P<0.001$), and a significant interaction between groups and shear stress ($P<0.001$). The control group was significantly different from the Tiron group ($P=0.02$) and the SOD/cat group ($P=0.04$, Tukey test). Thus, scavenging ROS significantly reduced the constriction that was due to luminal shear stress. Flows required to achieve a shear stress of 50 dyne/cm^2 were 108±19 μL/min for the control group (n=8), 144±22 μL/min for the Tiron group (n=6), and 190±20 μL/min for the SOD/cat group (n=6).

Figure 2 shows the effects of SOD/cat treatment on the shear stress–induced constriction in MCAs after the endothelium had been removed by the passage of air through the lumen (see Methods). Removal of the endothelium was verified by the absence of a dilation to luminally applied ATP. The absolute diameters are shown in Figure 2A, and the percent changes of the diameters as a function of shear stress are shown in Figure 2B. Even after the removal of the endothelium, MCAs significantly constricted to the luminal shear stress ($P=0.001$ and $P=0.03$ for absolute change [Figure 2A] and percent change [Figure 2B], respectively).

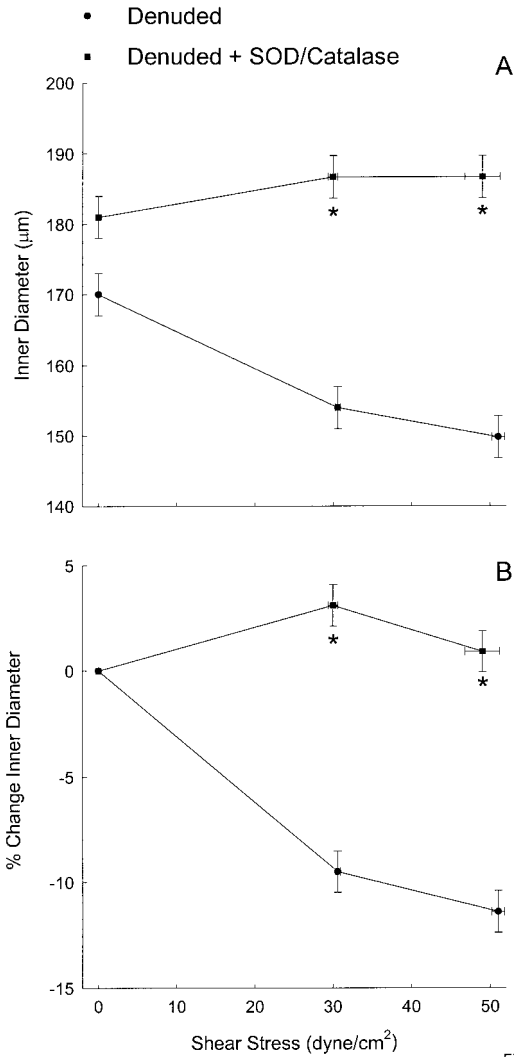

Figure 2. A, Effect of SOD (200 U/mL)/catalase (140 U/mL), scavenger of ROS, on the shear stress–induced constriction of the rat MCA after removal of the endothelium (see Methods). Removal of endothelium was verified by the absence of a dilation to luminally applied ATP. There was not a significant shear stress effect ($P=0.055$), but there was a significant group difference ($P=0.028$) and a significant interaction between the treatment groups and shear stress ($P<0.001$, 2-way repeated-measures ANOVA). The number of observations was 6 for each group. *$P<0.05$ compared with the corresponding diameter of the denuded (control) group (Tukey test). B, Same data as shown in panel A, except that the diameters were plotted as a percent change from the resting diameter (no shear stress, or 0 dyne/cm^2). There was a significant group effect ($P=0.004$), no significant shear stress effect ($P<0.061$), and no significant interaction between groups and shear stress ($P=0.92$). *$P<0.05$ compared with the corresponding diameter of the control group (Tukey test).

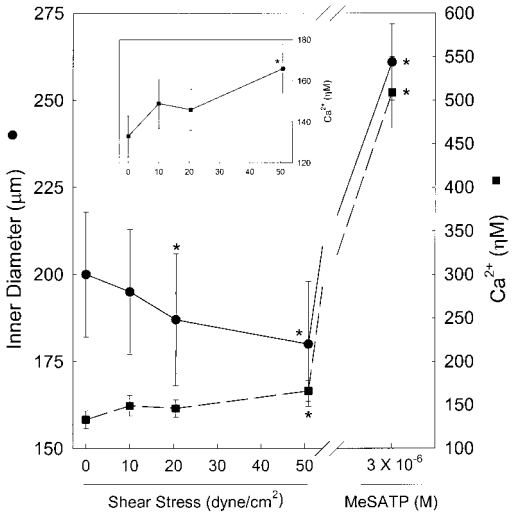

Figure 3. Effects of shear stress on MCA diameter (circles and left ordinate) and endothelial Ca^{2+} (squares and right ordinate). Diameter and endothelial Ca^{2+} were measured simultaneously from each MCA. Also shown on the right side of the plot are the responses of the same vessels to MeSATP, a P_2 agonist that dilates MCAs by stimulating the synthesis of NO. The insert shows an enlarged plot of the Ca^{2+} change in the endothelium with increasing shear stress. Repeated-measures ANOVA revealed a significant change in diameter and Ca^{2+} to shear stress ($P<0.001$ and $P=0.01$, respectively; n=5). *$P<0.05$ compared with corresponding values with no flow (0 dyne/cm^2).

After removal of the endothelium, SOD/cat completely abolished the shear stress–induced constriction in endothelium-denuded MCAs.

The effect of shear stress on diameter and endothelial Ca^{2+} (measured simultaneously from each MCA) is shown in Figure 3. The inside diameter progressively decreased from 200±18 to 180±18 μm (n=5, $P<0.001$) when the shear stress was increased from 0 to 50 dyne/cm^2. In the same MCAs, endothelial Ca^{2+} increased ≈33 nmol/L, from 133±10 to 166±12 nmol/L, over the same shear stress range ($P=0.01$). The insert in Figure 3 shows an enlarged plot of the Ca^{2+} change in the endothelium with increasing shear stress. Figure 3 also shows the diameter and Ca^{2+} change when 3×10^{-6} mol/L MeSATP was administered luminally. MeSATP dilates rat MCAs through the activation of NO synthase by increasing endothelial Ca^{2+}.[18,21] The 33-nmol/L increase in Ca^{2+} as a result of shear stress (50 dyne/cm^2) is considerably less than the increase of 376 nmol/L that resulted when the MCAs were dilated after the addition of MeSATP (Figure 3). Using data from a previous study, we estimate that a 33-nmol/L increase in endothelial Ca^{2+} could dilate MCAs through the stimulation of NO synthase by only 2% or less (S.P. Marrelli, unpublished data, 2000, and Marrelli[21]).

The effect of removing the endothelium on the shear stress–induced constriction is shown in Figure 4. Increasing luminal shear stress by increasing flow through the lumen constricted intact MCAs (Figure 4A) (n=4, $P<0.001$). After the flow was stopped and the MCAs were allowed to dilate, a similar response to shear stress was repeated (Figure 4A). The insert in Figure 4A expresses the data as percent change in the diameter of the MCAs, where the baseline was the inner diameter without flow (0 dyne/cm^2). There was no significant difference between the first response (control) and the second response (time control). After an initial response to shear stress in another group of MCAs, air was passed through the lumen of each vessel to damage the endothelium. Because damaging the endothelium significantly constricted the cerebral vessels, SNAP, an NO donor, was added to the extraluminal bath to dilate the vessel to near its original diameter before removal of the endothelium. After damage to the endothelium, the response of the MCAs to shear stress was enhanced ($P=0.04$, n=5) (Figure 4B), indicating that the endothelium attenuated the constrictor response to the shear stress.

Figure 5 shows the effects of 10^{-5} mol/L L-NAME (NO synthase inhibitor) alone or in combination with 10^{-5} mol/L indomethacin (cyclooxygenase inhibitor) on the constriction to luminal shear stress. In each of the panels, there was an initial control response (solid bars) during which luminal shear stress was adjusted to 30 dyne/cm^2. After returning the shear stress to 0 dyne/cm^2 by stopping flow, shear stress was adjusted to 50 dyne/cm^2 for ≈5 minutes, and the shear stress was again removed. The experimental condition (or time control) was then imposed, and the response to a shear stress of 30 and 50 dyne/cm^2 was repeated as described above (stippled bars in Figure 5). SNAP was added to each vessel in the experimental condition to restore the diameter to near the original baseline, because removal of the endothelium or

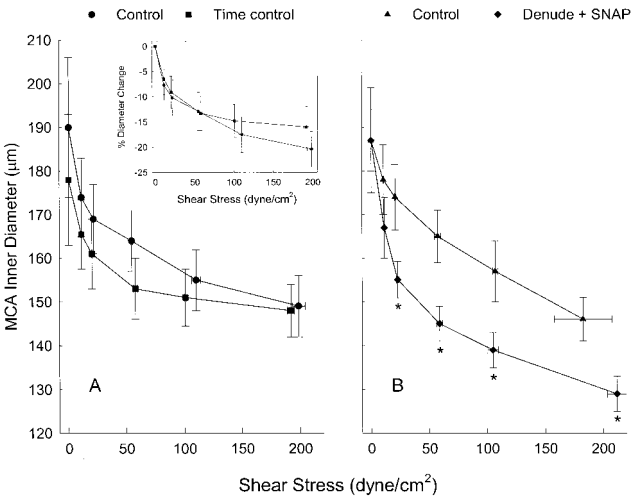

Figure 4. A, Effects of luminal shear stress (produced by increasing flow) on inside diameter in rat MCAs in control (first response) and time control (second response). Insert shows the data when plotted as percent change in inside diameter. There was a significant shear stress response ($P<0.001$, n=4) but no significant difference between the control and time control. B, Effects of luminal shear stress on inside diameter in rat MCAs in the control condition and after removal of the endothelium (denudation). Because the MCAs constricted after removal of the endothelium, SNAP, an NO donor, was added to dilate the vessels to near the original diameter before removal of the endothelium. Repeated-measures ANOVA revealed that there was a significant group response ($P=0.04$ for control vs denude+SNAP, n=5) and a significant response to shear stress ($P<0.001$). *$P<0.05$ compared with corresponding control value (Tukey test).

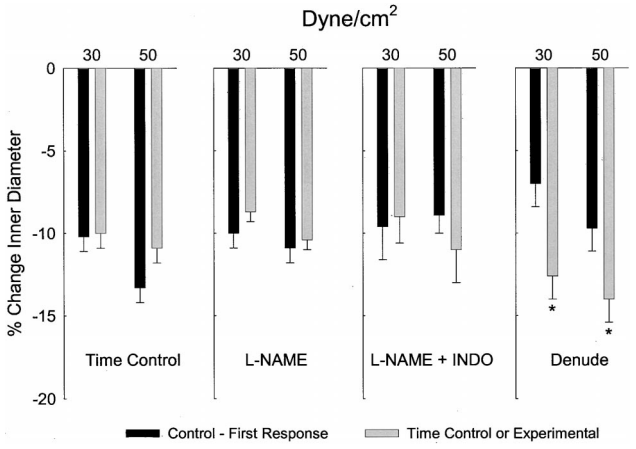

Figure 5. Effects of 10^{-5} mol/L L-NAME, an inhibitor of NO synthase, alone or in combination with 10^{-5} mol/L indomethacin (INDO), an inhibitor of cyclooxygenase, or damage to the endothelium (denudation) on the shear stress response in rat MCAs (n=8, 9, and 8, respectively). A time control is also included (left, n=8). SNAP, an NO donor, was added to each vessel in the experimental condition to restore the diameter to near the original baseline. *$P<0.05$ compared with corresponding initial or control response.

L-NAME treatment constricted the MCAs by ≈15%. From left to right, the panels in Figure 5 are as follows: the time control (n=8), the response in the presence of 10^{-5} mol/L L-NAME (n=9), the response in the presence of 10^{-5} mol/L L-NAME and 10^{-5} mol/L indomethacin (n=8), and the response after damage to the endothelium (denude) (n=8). The only condition showing a significant effect was after damage to the endothelium ($P=0.004$). Figure 6 shows a similar study in which the experimental condition was L-NAME+indomethacin+charybdotoxin (100 nmol/L) (n=6 each for time control and experimental groups). Charybdotoxin is an inhibitor of the Ca^{2+}-activated K^+ channels. SNAP was added to each vessel in the experimental

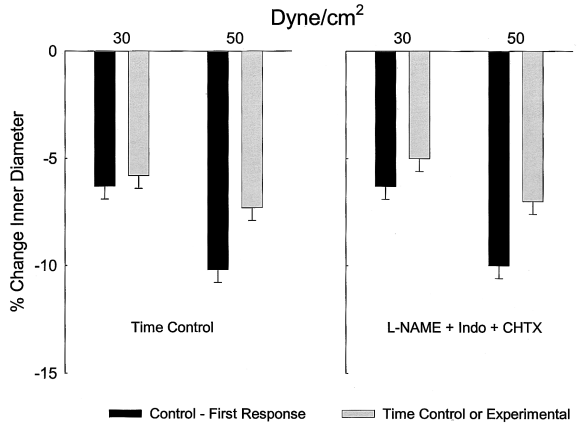

Figure 6. Effects of 10^{-5} mol/L L-NAME, an inhibitor of NO synthase, 10^{-5} mol/L indomethacin (Indo), an inhibitor of cyclooxygenase, and 100 nmol/L charybdotoxin (CHTX), an inhibitor of Ca^{2+}-activated K^+ channels, on the shear stress response in rat MCAs (n=6 each for time control and experimental group). SNAP, an NO donor, was added to each vessel in the experimental condition to restore the diameter to near the original baseline.

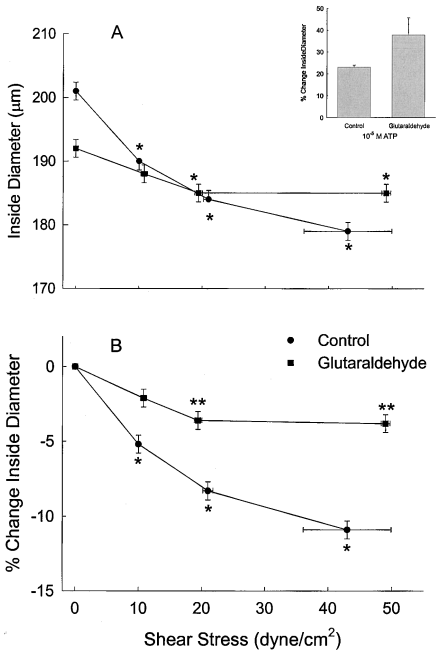

Figure 7. A, Effects of increasing the rigidity of endothelial cells (20-second luminal exposure to 0.025% glutaraldehyde) on the MCA diameter when luminal shear stress was increased. There was no significant group effect ($P=0.9$, n=4 for each group); however, there was a significant shear stress response ($P<0.001$) and a significant interaction between glutaraldehyde treatment and shear stress ($P<0.001$). Although glutaraldehyde treatment attenuated the constrictor response, it did not diminish the endothelial response to 10^{-5} mol/L ATP, indicating that the dilator function of the endothelium was intact (insert in panel A). B, Percent change in diameter in the control condition and after glutaraldehyde treatment when the luminal shear stress was increased (same data as in panel A). There was a significant group effect ($P=0.015$, n=4 for each group), a significant shear stress response ($P<0.001$), and a significant interaction between glutaraldehyde treatment and shear stress ($P<0.026$). *$P<0.05$ compared with no luminal shear stress (0 dyne/cm^2) for the corresponding group; **$P<0.05$ compared with corresponding changes in control MCAs.

condition to restore the diameter to near the original baseline. As the studies show in Figure 5, the presence of L-NAME+indomethacin+charybdotoxin did not affect the response compared with the control response. There was a tendency in the study shown in Figure 6 for the second response (time control) to be diminished compared with the original response. A similar finding applied to the time control and L-NAME groups in Figure 5.

Figure 7 shows the effects of increasing the rigidity of endothelial cells with a 20-second luminal exposure to 0.025% glutaraldehyde[20] (n=4 for each group) on the constrictor response to luminal shear stress. Although the MCAs still constricted to increased shear stress after glutaraldehyde treatment, the response was not as prominent as the response in control vessels (Figure 7A, statistical interaction between glutaraldehyde treatment and shear stress, $P<0.001$). Figure 7B shows the response in the control condition and after glutaraldehyde treatment when the diameter was plotted as percent change. Although glutaraldehyde treatment attenuated the constrictor response, it did not diminish the endothelial response to 10^{-5} mol/L ATP, indicating that the dilator function of the endothelium was intact (see insert).

Discussion

Previous studies have demonstrated that luminal flow (shear stress) constricted MCAs from the cat and rat and penetrating arterioles from the rat.[5,6] Additionally, flow constricted rabbit cerebral vessels during some but not all conditions.[7,8] The majority of the constriction occurred over the range of shear stress (<50 dyne/cm^2) experienced during normal physiological conditions.[22] The mechanism for the constriction appears to involve integrin binding (specifically, β_3 integrin), depolarization of the vascular smooth muscle, and an increase in cytoplasmic Ca^{2+}.[5,6] Flow-induced constrictions persisted after removal of the endothelium.[5,6] This observation may not seem logical on initial consideration because the cells receiving the mechanical stimuli were not required for the response to occur. In a previous paper, we speculated that the forces at the luminal surface of the endothelium are transmitted through the cytoskeletal matrix to mechanoreceptors on the extraluminal side of the endothelium.[5,23]

Although the endothelium is not needed for the shear stress–induced constrictions in cerebral vessels, it was not known whether the endothelium enhances, attenuates, or does not affect rat cerebral arteries. Therefore, the purpose of the present study was to better define the role of the endothelium in shear stress–induced constrictions. We tested the hypotheses that (1) ROS that are produced by the endothelium as a result of shear stress either attenuate or enhance the shear stress–induced constriction, and (2) the endothelium attenuates the shear stress–induced constriction by releasing NO, prostacyclin, or EDHF. From these studies, we report 2 findings: (1) ROS are involved in the shear stress–induced constriction in the rat MCA. (2) The endothelium attenuated the constrictor response to shear stress.

The first of our findings is the involvement of ROS in the shear stress–induced constriction in the rat MCA. The source of these ROS does not appear to be the endothelium. The rationale for testing the hypothesis was derived from previous observations that endothelial cells in culture generate ROS when exposed to shear stress.[9–11] ROS have been reported to either dilate or constrict cerebral vessels.[6,12,13] Furthermore, a previous study implicated ROS in the shear stress response, but the source (endothelium or vascular smooth muscle) was not determined.[6] Our studies also indicated that ROS were involved and that their source was not the endothelium. We base this conclusion on the observations that shear stress–induced constrictions were attenuated with ROS scavengers not only in endothelium-intact MCAs (Figure 1) but also in endothelium-denuded MCAs (Figure 2). Because ROS were generated in the absence of endothelium, it logically follows that the endothelium was likely not the source. Thus, the endothelium does not attenuate or potentiate the shear stress–induced constriction by generating ROS. Although ROS seem to be involved (Madden and Christman[6] and the present study), they do not appear to be generated by the endothelium; the apparent source of the ROS is the vascular smooth muscle.

The second of our findings is that the endothelium attenuates the constrictor response to shear stress. The attenuation was not due to the release of NO, prostacyclin, or EDHF. Determination of whether the endothelium enhances, attenuates, or does not affect the shear stress–induced constriction has not been straightforward.[5,6] In general, the strategy to assess the role of endothelium is to compare the response for a given condition (shear stress in this case) in endothelium-intact cerebral vessels with the response to the same condition in endothelium-denuded vessels. However, removal of the endothelium constricts vessels ≈15% because of the concomitant removal of endothelium-derived NO. The contractile state of a vessel often dictates the magnitude of response for a stimulus. That is, vessels that are already partially constricted after removal of the endothelium may not constrict as much to luminal shear stress as do endothelium-intact vessels. To overcome this limitation, we added an NO donor, SNAP, in the present study to restore the original diameter before removal of the endothelium. When this was done, it was clear that the endothelium attenuated the constrictor response to luminal shear stress (Figures 4 and far right bar graph of Figure 5).

The attenuation (or dilator influence) of the endothelium on the shear stress–induced response was not due to the release of NO, prostacyclin, or EDHF from the endothelium (Figures 5 and 6). The results from the present study leave us with the following question: what endothelial component is responsible for the attenuated response? We offer 2 possible explanations to this question. First, there could be an unknown dilating factor being released from the endothelium or some unknown dilating process involving the endothelium. The dilator process would serve to offset and, thus, attenuate the constriction produced by the shear stress. Second, the mechanical force of the shear stress could have been attenuated as it was transmitted across the endothelium to the vascular smooth muscle. It is reasonable to believe that the mechanoreceptors responsible for the shear stress–induced constriction are on the vascular smooth muscle.[5,6] A direct stimulation of these mechanoreceptors by shear forces in endothelium-denuded MCAs would be more efficient than indirect stimulation when the endothelium is intact. Consistent with this idea is the observation that the constrictor response to luminal shear stress was attenuated after increasing the rigidity of the endothelium (Figure 7).[16] Given that dilator mechanisms involving the endothelium other than NO, prostacyclin, or EDHF are not known, we tentatively conclude that an attenuation of the mechanical forces across the endothelium could account for the attenuated shear stress–induced constriction by the endothelium.

We also demonstrated that shear stress on the endothelium increased cytoplasmic Ca^{2+} in endothelium by 33 nmol/L (from 133 nmol/L at rest to 166 nmol/L at 50 dyne/cm^2) (Figure 3). From previous studies, we calculate that this increase in endothelial Ca^{2+} would have only minor effects, if any, in producing dilations through the release of NO or EDHF from the endothelium[21] (S.P. Marrelli, unpublished data, 2000). However, it must be noted that in peripheral vessels shear stress can stimulate NO release through a pathway not directly involving Ca^{2+} as a second messenger.[24] Thus, the absence of a major increase in endothelial Ca^{2+} does not rule out the involvement of an endothelial relaxing factor. However, the minor increase in endothelial Ca^{2+} (Figure 3) in combination with the studies in which inhibitors

were used (Figures 5 an 6) does rule out the involvement of known relaxing factors in attenuating the shear stress–induced contraction.

In summary, we have shown that the generation of ROS can account for at least part of the constriction in rat MCAs as a result of shear stress. The endothelium does not appear to be the source of the ROS. By a process of elimination, the vascular smooth muscle is the likely source. The endothelium attenuates the shear stress–induced constriction. Although shear stress increases cytoplasmic Ca^{2+} in the endothelium, the Ca^{2+} increase is not sufficient to stimulate the release of NO, prostacyclin, or EHDF.

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