Vascular Smooth Muscle Actin Cytoskeleton in Cerebral Artery Forced Dilatation

Marilyn J. Cipolla, PhD; George Osol, PhD

Background and Purpose—We investigated the role of actin polymerization in regulating arterial diameter in response to increasing pressure and modulating forced dilatation of cerebral arteries at pressures above the upper limit of autoregulation.

Methods—Posterior cerebral arteries (n=12) were isolated and pressurized in a special arteriograph that allowed control of intravascular pressure and measurement of lumen diameter. Intact arteries in the absence (control) or presence of 3.0 μmol/L cytochalasin B (CB), an inhibitor of actin polymerization, were subjected to stepwise increases in pressure from 75 to 200 mm Hg. Lumen diameter was continuously recorded, as was the pressure at which forced dilatation (loss of tone) occurred. After a period of time at 200 mm Hg, pressure was returned to 75 mm Hg and the extent of tone recovery was evaluated.

Results—Arteries with and without CB developed a similar amount of tone during equilibration at 75 mm Hg: percent tone=27±3% for control versus 29±4% for CB arteries (P>0.05). However, arteries in the presence of CB could not withstand pressure as well and underwent FD at significantly lower pressures: 168±5 mm Hg for control versus 142±5 mm Hg for CB arteries (P<0.01). The amount of tone that arteries regained after FD when pressure was returned to 75 mm Hg was also less in CB arteries: percent tone=34±3% for control versus 11±2% for CB arteries (P<0.01).

Conclusions—Cytoskeletal integrity appears important for maintaining cerebral arterial diameter during changing intravascular pressure. In addition, the process of actin polymerization may be a significant contributor to development of myogenic tone after forced dilatation. (Stroke. 1998;29:1223-1228.)

Key Words: actin cytoskeleton ■ cerebral arteries ■ forced dilatation ■ rats

The arteries and arterioles of the cerebral circulation are highly effective at maintaining cerebrovascular resistance and autoregulation of blood flow over a wide range of pressures, primarily as a result of the myogenic mechanisms of the surrounding VSM.1–4 Myogenic reactivity is the process by which VSM increases force production in response to elevated intravascular pressure to maintain diameter, thus contributing to autoregulation of cerebral blood flow.3,4 This process is thought to involve several mechanisms within VSM, including stretch-activated calcium channels, membrane potential, and intracellular signaling via protein kinase C and phospholipase C (for review, see Reference 5). However, in most nonmuscle cells, mechanotransduction of pressure into a cellular response occurs via the actin cytoskeleton.6–10 Pressure or stretch is sensed, and the cell responds with rapid polymerization of globular actin into filaments and stress fiber formation.6–10 Since VSM is phenotypically most similar to nonmuscle cells such as fibroblasts,11 in that it does not contain distinct striations or myofibrils, it is possible that the process of actin polymerization occurs in VSM in response to pressure and contributes to diameter regulation of cerebral arteries to elevated intravascular pressure.

See Editorial Comment, page 1228

While cerebral artery diameter is well maintained within the myogenic pressure range, acute increases in blood pressure beyond the limit of autoregulation result in forced dilatation (FD) of cerebral vessels, autoregulatory breakthrough, and disruption of the blood-brain barrier.15–18 The consequence of these events is elevated arteriolar pressure and a marked increase in cerebral blood flow and vascular permeability, all of which contribute to the development of hypertensive encephalopathy.15–22 The vasodilation associated with FD in response to severe acute hypertension is usually ascribed to stretching of the vessels by the increased intravascular pressure, which overcomes the autoregulatory contraction of the VSM. This autoregulatory breakthrough of cerebral vessels has been shown in most23,24 but not all21 studies to be reversible once blood pressure is returned to normal; however, the mechanism of tone recovery as well as the process of FD is not known.

We hypothesized that a dynamic actin cytoskeleton contributes to arterial diameter regulation during changing intravascular pressure and that FD results in disruption of actin
filaments. Therefore, repolymerization of actin filaments may be necessary for tone recovery after FD. To test this, cannulated and pressurized cerebral arteries were subjected to increases in intraluminal pressure in the absence or presence of cytochalasin B (CB), a specific inhibitor of actin polymerization, to investigate the contribution of actin polymerization in modulating myogenic reactivity and FD.

Materials and Methods

Preparation of Arterial Segments

Male Wistar rats (n=12; B&K Universal, Fremont, Calif) weighing 270 to 320 g were used for all experiments. The rats were lightly anesthetized with ether and quickly decapitated, as approved by the Oregon Health Sciences University Animal Care Facility. After decapitation, the entire brain was removed and placed in cold (4°C) and oxygenated PSS. A third-order branch of the posterior cerebral artery (inner diameter =131±5 μm at 75 mm Hg), identified from specific anatomic location, was carefully dissected and cleared of connective tissue. Once excised, the arteries were transferred directly into the arteriograph chambers.

Pressurized Arteriograph and Measurement of Lumen Diameter

The arteriograph (Living Systems Instrumentation) consisted of two 10-mL chambers with inlet and outlet ports to allow for superfusion of the arteries with PSS and for application of drugs. The superfusate (PSS) was continually recirculated from a 50-mL reservoir and pumped with a peristaltic pump through a heat exchanger to warm the PSS to 37°C before it entered the arteriograph chamber. The PSS was aerated in the reservoir with a mixture of 10% O2/5% CO2/85% N2 to maintain a constant pH of 7.4±0.05.

Each of two arteriograph chambers contained a set of proximal and distal glass microcannulas (tip=50 μm) on which an artery was mounted, secured with single strands of nylon suture (diameter=10 μm), and perfused gently with oxygenated PSS. The proximal cannula was attached to an in-line pressure transducer and servomechanism that continually measured and adjusted TMP. The servo system consisted of a small peristaltic pump and controller that permitted TMP to either be maintained at a set pressure (static) or increased at a variable rate. In these experiments, the distal cannulas were closed off so that there was no flow through the vessels.

Once both arteries were cannulated and pressurized, the arteriograph was transferred to the stage of an inverted microscope with an attached video camera and monochrome monitor. Each cannulated artery was suspended on bulkheads just above an optical window in the bottom of the chamber, which allowed for viewing of the artery and electronic measurement of lumen diameter by a technique previously published. Briefly, the transilluminated image of the artery on the video monitor was used to electronically determine the dimensions of the artery by the video dimensional analyzer. The

---

**Selected Abbreviations and Acronyms**

- CB = cytochalasin B
- FD = forced dilatation
- ILV = indolactam V
- PSS = physiological saline solution
- TMP = transmural pressure
- VSM = vascular smooth muscle

---

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** A. Diameter and pressure tracings of an intact artery subjected to stepwise increases in intravascular pressure. Pressures above 125 mm Hg are shown, with FD occurring at 175 mm Hg. When pressure was returned to 75 mm Hg, the artery regained tone. B. Diameter and pressure tracings of an artery in CB (3.0 μmol/L) subjected to stepwise increases in TMP. Pressures above 100 mm Hg are shown, with FD occurring at 150 mm Hg. Note that little tone was restored when pressure was returned to 75 mm Hg.
Experimental Protocols

All arteries (n=12) for experimentation were equilibrated for 1 hour at 75 mm Hg, during which time spontaneous tone developed. After equilibration, pressure was increased in 25-mm Hg increments to 200 mm Hg. The diameter at each pressure as well as the pressure at which FD occurred was recorded. After the arteries were at 200 mm Hg for approximately 5 minutes, the pressure was lowered to 75 mm Hg. Arteries were left at 75 mm Hg for 20 to 30 minutes, and the diameter and the amount of tone recovered was recorded.

Several arteries (n=6) were given 3.0 μmol/L CB at 75 mm Hg after equilibration and approximately 5 minutes before pressure was increased. This concentration of CB did not alter baseline diameter. Pressure was then increased stepwise in these arteries, as described above. Since these arteries did not redevelop significant tone after FD once pressure was returned to 75 mm Hg, arteries were given 1.0 μmol/L ILV, a specific activator of protein kinase C and potent smooth muscle cell constrictor, to test for contractility and viability.

After each experiment, 0.1 mmol/L papaverine was applied to induce relaxation and obtain a fully relaxed diameter at each transmural pressure.

Data Calculations and Statistical Analysis

All results are presented as mean±SE. The amount of intrinsic tone an artery possessed was calculated as a percent decrease in lumen diameter from the relaxed diameter in 0.1 mmol/L papaverine. Differences between arteries with and without CB were determined with ANOVA. Differences before and after FD were determined with ANOVA with repeated measures and considered significant at P<0.05.

Drugs and Solutions

The perfusate and superfusate for all experiments consisted of a bicarbonate-based phosphate buffer (Ringer’s PSS), the composition of which was as follows (mmol/L): NaCl 119.0, NaHCO₃ 24.0, KCl 4.7, KH₂PO₄·H₂O 1.17, CaCl₂ 1.6, EDTA 0.026, and glucose 5.5. PSS was made fresh each week and stored without glucose at 4°C. PSS was made fresh each week and stored without glucose at 4°C. Glucose was added to the PSS before each experiment. CB was purchased from Calbiochem. A stock solution of 10⁻² mol/L CB in dimethylsulfoxide was mixed each week and stored at −20°C. ILV was purchased from LC Laboratories and mixed as 10⁻³ and 10⁻⁴ mol/L stock solutions each week. Papaverine was purchased from Sigma Chemical Co and mixed as a 10⁻² mol/L stock solution each week and stored at 4°C.

Results

Response of Arteries in the Absence and Presence of Cytochalasin B

Arteries in the presence of CB underwent FD at significantly lower pressures and regained considerably less tone than control arteries without CB. The diameter and pressure tracings shown in Figure 1A demonstrate the typical response of a control artery to elevated pressure. This vessel developed intrinsic tone during equilibration and maintained a constant diameter of 120 μm from 75 to 125 mm Hg. When pressure was increased to 150 mm Hg, the artery began to dilate to the elevated distending pressure and underwent FD (lost tone) when pressure was increased to 175 mm Hg. Additional increases in pressure caused further dilatation and increased diameter, thus demonstrating a lack of myogenicity at pressures beyond the myogenic range. When pressure was returned to 75 mm Hg, the artery regained tone to a greater extent than before the pressure was increased, and diameter was below baseline at 105 μm.

Figure 1B demonstrates the behavior of arteries when actin polymerization was inhibited in the presence of CB. Arterial dilation began at 125 mm Hg, and FD occurred when pressure was increased to 150 mm Hg. This artery regained little tone when pressure was returned to 75 mm Hg. The amount of tone present in each artery type before and after FD, as well as the pressure at which FD occurred, is shown in the Table.

Pressure-Diameter Response: Effect of Cytochalasin B

The presence of CB did not affect basal diameter, which was 131±4 μm in control and 125±9 μm in CB arteries (P>0.05) after equilibration at 75 mm Hg. In addition, the amount of tone was similar between the groups at 75 mm Hg before pressure was increased: percent tone=27±3% for control arteries and 29±4% for CB arteries (P>0.05). However, arteries in the presence of CB could not withstand pressure as well when it was increased to 200 mm Hg, and they underwent FD at significantly lower pressures. FD occurred at 168±5 mm Hg for control versus 142±5 mm Hg for CB arteries (P<0.01), as shown in the Table. Figure 2 shows the pressure-diameter response of arteries in the absence and presence of CB.

![Figure 2. Diameter versus TMP for intact arteries in the absence and presence of CB (3.0 μmol/L). **P<0.01.](http://stroke.ahajournals.org/doi/10.1161/01.STR.80.6.1225)
Extent of Tone Recovery After FD: Effect of Cytochalasin B

After FD, when pressure was returned to 75 mm Hg, control arteries regained a somewhat higher, but not statistically significant, level of intrinsic tone than before pressure was increased (Table); however, arteries in the presence of CB regained little tone when pressure was returned to 75 mm Hg. The amount of tone recovered at 75 mm Hg after FD was 34±6% for control (P<0.05 versus control before FD) and 11±6% for CB arteries (P<0.01 versus control after FD and versus CB before FD).

Control for the Effect and Specificity of Cytochalasin B

To determine whether arteries in CB that did not regain tone after FD were not damaged or rendered incapable of constricting in CB, arteries were given 1.0 μmol/L ILV after FD. Addition of this compound constricted arteries in CB by 38±6%. However, when pressure was then increased in these arteries, they could not withstand pressure and dilated. The diameter and pressure tracings shown in Figure 3A demonstrate this behavior. This artery was initially in PSS and developed tone on increasing pressure greater than 50 mm Hg. FD occurred at 150 mm Hg, after which tone was restored when pressure was returned to 75 mm Hg. The artery was then given 3.0 μmol/L CB, which did not alter baseline diameter, but when pressure was increased again, the artery underwent FD at 125 mm Hg and regained little tone once pressure was returned to 75 mm Hg. Although this artery developed little pressure-dependent tone after FD, addition of ILV (1.0 μmol/L) caused contraction in the presence of CB. However, when pressure was then increased to 100 mm Hg, the artery could not maintain diameter and dilated to the elevated intravascular pressure. A graph of the percent constriction of intact arteries at 75 mm Hg in the presence of CB before FD, after FD, after FD contracted in 1.0 μmol/L ILV, and after FD in ILV after pressure was increased to 100 mm Hg is shown in Figure 3B.

Discussion

The present study demonstrates that the highly effective regulation of cerebral artery diameter in response to changes in intravascular pressure (myogenic reactivity) is altered in the presence of CB, an inhibitor of actin polymerization.25,26 This was shown by the finding that arteries in the presence of CB, which had an amount of basal tone similar to that of control arteries, underwent FD at significantly lower pressures than arteries without CB. In addition, arteries without CB regained myogenic tone after FD when pressure was returned to 75 mm Hg; however, this behavior did not occur in the presence of CB, suggesting that actin polymerization may be an important mediator of myogenic activity and cerebral artery responses to pressure.

In nonmuscle cells, the actin cytoskeleton is a dynamic structure that responds to mechanical stimuli such as tension with polymerization of monomeric globular (G-) actin into filamentous (F-) actin, thereby increasing the number of actin cables.6–10 It is possible that, similar to nonmuscle cells, the actin cytoskeleton of VSM also responds to pressure with polymerization of monomeric actin stores into filaments, thus contributing to myogenic reactivity. We therefore hypothesized that arteries in which actin polymerization was inhibited would have a diminished capacity to regulate diameter. In addition, FD would result in disruption of the actin cytoskeleton, and repolymerization of actin filaments would be necessary for recovery of tone after FD. Since arteries in the
presence of CB had decreased reactivity to pressure (Figures 1 and 2) and regained significantly less tone after FD when pressure was returned to 75 mm Hg (Table), the process of actin polymerization as a mechanism of increasing force production in VSM in response to pressure appears likely.

In a related study, we determined that induction of actin polymerization by jasplakinolide, a cell-permeable inducer of actin polymerization, produced contraction and increased the level of tone in cerebral arteries by 29%. In addition, arteries at a pressure of 125 mm Hg that had diameters similar to those of arteries at 75 mm Hg (136 ± 2 versus 133 ± 11 μm) had considerably less G-actin content (as determined by fixing the arteries pressurized, staining for G-actin with DNase I, and viewing the arteries with the use of confocal microscopy), indicating a G- to F-actin transition in cerebral artery VSM in response to the higher pressure. Together with the present study, these results suggest that actin polymerization is a mechanism by which VSM can increase force production in response to pressure.

Furthermore, arteries in cytochalasin D had diminished reactivity to pressure (Figures 1 and 2) and regained significantly less tone after FD when pressure was returned to 75 mm Hg (Table), the process of actin polymerization as a mechanism of increasing force production in VSM in response to pressure appears likely.

A few studies have also investigated the dynamic nature of the actin cytoskeleton in smooth muscle. Mauss et al showed that inhibition of actin polymerization by Clostridium botulinum C2 toxin (which ADP-ribosylates monomeric G-actin) impaired the contraction of smooth muscle isolated from guinea pig ileum. This effect was shown to be caused by a direct action of the toxin on the smooth muscle. Because filamentous F-actin is not a substrate for C2 toxin, these findings provide evidence for a role of G- to F-actin transition in smooth muscle contraction. In a similar study, when actin polymerization was blocked by CB, K+-induced contraction of intestinal smooth muscle cells was inhibited in a dose-dependent manner, without any significant effect on voltage-dependent calcium channels, membrane potential, or myosin light chain phosphorylation, indicating an influence of actin assembly on smooth muscle contraction.

The present study, however, suggests for the first time that myogenic responses (ie, pressure-dependent contraction) of cerebral arteries was diminished when actin polymerization was blocked by CB.

Cytochalasins have been shown to have other cellular effects in addition to blocking actin polymerization, including inhibiting glucose transport and promoting depolymerization of actin microfilaments. We do not believe that inhibition of glucose transport by CB is significant in this study because we have observed that arteries can maintain tone in the absence of exogenous glucose in excess of 2 hours (M.J.C., unpublished data, 1995). The possibility that CB may be causing depolymerization of actin filaments is also unlikely considering that at this concentration of CB, baseline arterial diameter was unaltered and arteries maintained greater than 30% tone. In a related study, we determined that high concentrations (>50 μmol/L) of cytochalasin D caused dilation and loss of tone in cerebral arteries. These arteries also had significantly greater G-actin content than arteries with tone, suggesting that dilation is associated with depolymerization of actin filaments. These arteries in cytochalasin D not only lost tone but also could not contract to agonist stimulation, including ILV. In contrast to the present study, arteries in a low concentration of CB maintained tone and contracted to ILV, suggesting that CB was inhibiting polymerization but not causing significant depolymerization of actin filaments.

The concentration of CB used (3.0 μmol/L) did not alter the baseline diameter of these arteries, which had diameters and levels of tone similar to those of control arteries without CB. Figure 2 shows that the diameter of arteries was 131 ± 4 μm for control and 125 ± 9 μm for CB arteries (P > 0.05) after equilibration at 75 mm Hg. This was not a statistically significant difference and likely not biologically significant either since the amount of basal myogenic tone was also similar between the groups (27 ± 3% for control and 29 ± 4% for CB arteries; P > 0.05). This is an important consideration since arteries that are more or less contracted would have a different wall tension and therefore may respond differently to pressure. Along these lines, another group of arteries were denuded of endothelium (data not shown) and were more contracted than intact arteries, likely due to the loss of endothelium-derived nitric oxide. The diameter of these denuded arteries was 104 ± 6 μm at 75 mm Hg, and the basal myogenic tone was 42 ± 3%. It is interesting that these arteries could much better withstand pressure than intact arteries and underwent FD at 191 ± 5 mm Hg. This was not the case for denuded arteries in the presence of CB. These arteries were more contracted (diameter = 93 ± 10 μm; percent tone = 53 ± 4%) but underwent FD at much lower pressures (158 ± 5 mm Hg). Therefore, it appears that under normal conditions, a more contracted state protects from overdistension and FD, but when actin polymerization is inhibited by CB, myogenicity is diminished. While the role of the endothelium in this response is not apparent, it is clear that both intact and denuded arteries in the presence of CB had diminished reactivity to pressure and underwent FD at lower pressures, further suggesting a role for actin polymerization in mediating myogenic responses.

In conclusion, cerebral artery reactivity to pressure was diminished in the presence of CB, an inhibitor of actin polymerization. Arteries also underwent FD at significantly lower pressures, suggesting that a dynamic actin cytoskeleton is important for myogenic responses. In addition, arteries regained considerably less tone after FD when pressure was returned to 75 mm Hg, indicating that the process of actin polymerization is important for recovery of myogenic tone after FD.

Acknowledgments

This study was supported by American Heart Association Grant-in-Aid 93014090. We would like to acknowledge Kathi Derrickson and Nicole Bang of the Surgery Graphics Department at Oregon Health Sciences University for their expert assistance with the figures.
References

Editorial Comment

Pronounced, acute increases in arterial blood pressure induce severe and extensive changes in the cerebral vasculature. Such changes are associated with histological changes in the endothelium and vascular smooth muscle; marked, sometimes uneven, sustained vasodilation; increased permeability to macromolecules; and alterations in the reactivity of the vessels.1 These changes are partly mediated by generation of oxygen radicals, since they are either prevented or minimized by pretreatment with scavengers of these radicals.2 Interest in this phenomenon resides, in part, that it may be a model for human hypertensive encephalopathy.2

The preceding article by Cipolla and Osol reports evidence that the forced dilation of cerebral arteries from excessive intravascular pressure is influenced by treatment with cytochalasin B, an inhibitor of actin polymerization.

The vessels treated with this enzyme developed more pronounced changes in reactivity and displayed forced dilatation at lower pressures than controls. This is a clear indication that changes in the cytoskeleton influence vascular reactivity. Hopefully, this study will generate additional interest in the complex relationships between cytoskeletal integrity and function and vascular reactivity.

Hermes A. Kontos, MD, PhD
Associate Editor for Basic Science
School of Medicine
Medical College of Virginia
Richmond, Virginia

References
Vascular Smooth Muscle Actin Cytoskeleton in Cerebral Artery Forced Dilatation
Marilyn J. Cipolla and George Osol

Stroke. 1998;29:1223-1228
doi: 10.1161/01.STR.29.6.1223
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/29/6/1223

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/