Vascular smooth muscle cell stress as a determinant of cerebral artery myogenic tone

JOHAN FREDRIK BREKKE,¹ NATALIA I. GOKINA,² AND GEORGE OSOL²
¹Department of Physiology, University of Bergen, Bergen, Norway; and ²Department of Obstetrics and Gynecology, University of Vermont, Burlington, Vermont 05405

Received 22 July 2002; accepted in final form 14 August 2002

Brekke, Johan Fredrik, Natalia I. Gokina, and George Osol. Vascular smooth muscle cell stress as a determinant of cerebral artery myogenic tone. Am J Physiol Heart Circ Physiol 283: H2210–H2216, 2002; 10.1152/ajpheart.00633.2002.—Although the level of myogenic tone (MT) varies considerably from vessel to vessel, the regulatory mechanisms through which the actual diameter set point is determined are not known. We hypothesized that a unifying principle may be the equalization of active force at the contractile filament level, which would be reflected in a normalization of wall stress or, more specifically, media stress. Branched segments of rat cerebral arteries ranging from <50 μm to >200 μm in diameter were cannulated and held at 60 mmHg with the objectives of: 1) evaluating the relationship between arterial diameter and the extent of myogenic tone, 2) determining whether differences in MT correlate with changes in cytosolic calcium ([Ca²⁺]i), and 3) testing the hypothesis that a normalization of wall or media stress occurs during the process of tone development. The level of MT increased significantly as vessel size decreased. At 60 mmHg, vascular smooth muscle [Ca²⁺], concentrations were similar in all vessels studied (averaging 230 ± 9.2 nM) and not correlated with vessel size or the extent of tone. Wall tension increased with increasing arterial size, but wall stress and media stress were similar in large versus small arteries. Media stress, in particular, was quite uniform in all vessels studied. Both morphological and calcium data support the concept of equalization of media stress (and, hence, vascular smooth muscle cell stress and force) as an underlying mechanism in determining the level of tone present in any particular vessel. The equalization of active (vascular smooth muscle cell) stress may thus explain differences in MT observed in tone in different-sized vessels. A corollary to this hypothesis is that force production by VSM cells may be related to the structure of the vessel, specifically, to VSM mass. More specifically, we tested the hypothesis that media stress may be normalized and that this property, which more closely approximates VSM cell stress, may account for some of the variability observed in tone in different-sized vessels. A corollary to this hypothesis is that force production by VSM cells in response to a pressure stimulus is uniform in different-sized arteries. Although it is difficult to measure active force directly in a cannulated preparation, we used the dual approach of: 1) calculating wall and media stress based on morphometric measurements obtained via light and electron microscopy, and 2) directly measuring intracellular calcium concentrations in different-sized vessels with different degrees of tone.

METHODS

Preparation of vessels and calculations. Two series of experiments were conducted in this study, with measurement performed on 23–24 vessels in each. MT and smooth muscle...
intracellular calcium concentrations ([Ca\(^{2+}\)]) were determined in series 1, whereas circumferential tension and wall and media stress were determined in series 2. This approach was used because stress calculations involved fixation and processing for transmission electron microscopy of vessels with tone, hence, fully relaxed diameters could not be obtained due to fixation, requiring an additional set of vessels. In all cases, vessels were obtained from the brains of normotensive male Wistar-Kyoto rats (14–16 wk of age, n = 21) anesthetized with Brevital Sodium (50 mg/kg) on the day of the experiment and decapitated with a small animal guillotine. The posterior cerebral artery (PCA) with intact branches was dissected free from connective tissue in HEPES-PSS and cannulated within the experimental chamber of an arteriograph. Once cannulated, all branches were tied off with surgical sutures, and the absence of leakage was verified by the maintenance of a stable pressure. The PCA preparation was equilibrated for 1 h (37°C, pH 7.4, 10 mmHg) before experimentation. Lumen diameter (D), wall thickness (w), and transmural pressure were measured with the use of a Nikon TMS inverted microscope and the Living Systems Instrumentation (Burlington, VT) pressurized vessel system.

At the beginning of an experiment in series 1, intraluminal pressure was increased from 10 to 60 mmHg, stimulating the development of MT. In series 2, D and w values were collected from all branches in the presence of tone by taking measurements from branch images on the screen of a television monitor at an optical magnification of \(1256\) before fixation. Vessels were then fixed for transmission electron microscopy under pressure (60 mmHg) as described below.

In series 1, maximal diameter (\(D_{\text{max}}\)) was obtained in the presence of 0.1 mM papaverine, which eliminates MT and induces full relaxation. MT was calculated as

\[
\text{MT}(\%) = \frac{(D_{\text{max}} - D)/D_{\text{max}}}{100}
\]

Wall tension (T) was determined by using the Laplace formulation \(T = \rho R \sqrt{\pi} \), where \(\rho\) is transmural pressure (in dyn/cm\(^2\)) and \(R\) is radius (in cm), measured at the midpoint of the arterial wall, to yield tension (as dyne/cm)]. Stress (\(\delta\)), defined as the force exerted on a material divided by the area over which the force is exerted (in the deformed state, i.e., “true” or Cauchy stress) was calculated based on the Laplace formulation. For wall stress, this was defined as \(\delta_w = T/\omega\) (expressed in dyn/cm\(^2\) \(\times 10^9\)).

Media thickness was determined by electron microscope imaging of the vessel wall in individual branches. After development and stabilization of MT, diameter and wall thickness were recorded in each branch. The PCA segment was then fixed by addition of 200 \(\mu\)l of 2.5% glutaraldehyde solution directly into the experimental chamber (30 min). Vessels were observed during fixation to verify that the diameter was not altered during the process. Arterial segments were subsequently transferred to Millonig’s solution for storage before processing. PCA preparations were sectioned longitudinally (parallel to the long axis of the vessel) for determination of media thickness and analysis of VSM cell content. Care was taken to use only orthogonal sections based on visual examination of the sectioned block and section thickness (only the thinnest sections used). Measurements of media thickness were made from black and white photographs of sections from different-sized vessels. This information (combined with the lumen diameter and constant transmural pressure of 60 mmHg) at the time of fixation was used to calculate media stress.

Measurement of [Ca\(^{2+}\)]. For measurements of intracellular smooth muscle cell [Ca\(^{2+}\)], PCA segments were mounted in the experimental chamber of an arteriograph and pressurized to 10 mmHg. Autofluorescence and background fluorescence were determined, and arteries were then loaded with the ratiometric, Ca\(^{2+}\)-sensitive fluorescent dye fura 2-AM (5 \(\mu\)M, initially diluted with pluronic acid to assure fura 2 solubility) for 30 min at room temperature. After loading was completed, vessels were continuously superfused with PSS buffer gassed with 10% \(\text{O}_2\)-5% \(\text{CO}_2\) balance \(\text{N}_2\) (pH 7.4) at 37°C for an additional 45 min. Intraluminal pressure was increased from 10 to 60 mmHg, stimulating the development of MT. Fura 2 fluorescence was measured by using a photomultiplier system (IonOptix; Milton, MA). Background- and autofluorescence-corrected emission ratios (510 nm), recorded from all individual branches alternatively excited at 340 and 380 nm, were obtained at a sampling rate of 5 Hz by using IonWizard software (IonOptix).

Arterial wall [Ca\(^{2+}\)] was calculated by using the following equation: \([\text{Ca}^{2+}]_i = K_C(\beta - R_{\text{min}})/(\beta - R),\) where \(R_{\text{min}}\) and \(R_{\text{max}}\) are minimum and maximum ratio, respectively, which were measured in separate experiments from ionomycin-treated arteries, and \(\beta\) was determined as previously described (12).

The values were then pooled and used to convert the ratio values into a [Ca\(^{2+}\)]\(_i\). The dissociation constant was 282 nM as determined by using in situ titration of Ca\(^{2+}\) in fura 2-loaded posterior cerebral arteries (18).

Solutions and drugs. The composition of HEPES-PSS was (in mM) 142 NaCl, 4.7 KCl, 1.7 MgSO\(_4\), 0.5 EDTA, 2.8 CaCl\(_2\), 1.2 K\(_2\)HPO\(_4\), 10 HEPES, and 5 glucose. The physiological salt solution (PSS) contained (in mM) 119 NaCl, 4.7 KCl, 23.8 NaHCO\(_3\), 1.1 KH\(_2\)PO\(_4\), 0.026 EDTA (disodium), 1.18 MgSO\(_4\), 10 glucose, and 1.6 CaCl\(_2\). Fura 2-AM and pluronic acid were purchased from Molecular Probes (Eugene, OR). All other chemicals were purchased from Sigma Chemical (St. Louis, MO) and solutions prepared on a daily basis.

Statistical analysis. Because of the nature of the PCA preparation, in this study the n value for each protocol represents total number of branches. Typically, each cannulated PCA preparation contained from two to five branches. The number of rats used for each protocol is therefore smaller than the given n value, which reflects the number of actual vessels studied.

Data were subjected to regression analysis, which yielded linear regression equations and correlation coefficients (\(r^2\) values) for tone, calcium, and stress (wall and media) as a function of arterial diameter. Tension was calculated as described above, although it deserves note that it was proportional to diameter because pressure was kept constant. To evaluate whether there were differences in a specific parameter (e.g., level of tone) based on arterial size, data were ranked according to vessel lumen diameter (with tone). Because there were \(\sim\)24 vessels in each series of experiments, we compared the 8 smallest versus the 8 largest branches to evaluate size-dependent differences in tone, calcium, tension, and stress. When large versus small vessels were compared, the Student’s t-test (unpaired) was used to compare differences between treatment means, with \(P\) values < 0.05 considered significant.

RESULTS

A typical cannulated PCA preparation, pressurized to 60 mmHg without intraluminal flow, is shown in Fig. 1. In this vessel, branches ranged from \(\sim\)40 to \(\sim\)200 \(\mu\)m in diameter. The relationship between lumen diameter and MT in rat PCA branches at 60 mmHg is shown in Fig. 2A. MT in the smallest branch (35 \(\mu\)m) was \(\sim\)46% and declined gradually as diameter in-
creased (27% in the largest branch, 209 μm). Regression analysis yielded the equation: MT = 53.8 – 0.124r, where r is radius, with a correlation coefficient of r² = 0.43. The level of tone was significantly greater in small versus large vessels (Fig. 2B).

The relationship between arterial lumen diameter and [Ca²⁺]i for individual vessel branches at 60 mmHg is summarized in Fig. 3A. As pressure was raised from 10 to 60 mmHg, [Ca²⁺]i increased significantly in all branches examined, an event associated with tone development. At 60 mmHg, [Ca²⁺]i was similar in all branches, regardless of variations in the extent of tone or lumen diameter, and averaged 230 ± 9.2 nM. The regression equation was [Ca²⁺]i = 196 + 0.24r, with a correlation coefficient of r² = 0.04. When vessels were grouped by size, there were no differences in [Ca²⁺]i (Fig. 3B).

Wall tension increased in direct proportion to arterial size (as expected, because of constant 60 mmHg pressure) and was significantly greater in the large versus small arterial group (289 ± 29.7 vs. 662 ± 29.4 dyn/cm, respectively, see Fig. 4).

Transmission electron microscopy images taken from the two extremes of the vessel branch range used in series 2 are shown in Fig. 5. Vessels were fixed at 60 mmHg and cut longitudinally to illustrate media thickness and wall ultrastructure. Figure 5A is from a small, 35-μm diameter vessel with a 4-μm thick media, whereas Fig. 5B shows a larger, 205-μm diameter vessel with a 14-μm thick media.

Wall stress, based on measurements made through the microscope, is shown as a function of vessel size in Fig. 6. The curve shows a progressive increase in wall stress with increasing arterial diameter. The regression equation was σw (wall stress) = 203.4 + 1.12r, with a correlation coefficient of r² = 0.41. When wall stress was compared in large versus small arteries (Fig. 6B), it was somewhat greater in larger vessels, although this difference did not attain statistical significance (P = 0.11).

The relationship between lumen diameter and media stress, based on analysis of transmission electron microscopy photographs, is shown in Fig. 7A. The regression equation for media stress was σm = (9.2 × 10⁵) – 663r, r² = 0.02. Media stress in large versus small arteries was virtually identical (Fig. 7B).

DISCUSSION

Earlier in vivo studies using the cat as an experimental model have examined the degree of cerebrovascular tone present in different-sized vessels and, in accordance with our results, reported an inverse relationship between the level of tone and arterial size (10, 11). One limitation of the in vivo approach is the inability to isolate the origins of the tone, because arterial diameter reflects an integration of numerous influences, e.g., endothelial, myogenic, humoral, metabolic, and neural. The isolated, pressurized vessel
methodology allows for the elimination of extrinsic influences and is a commonly used preparation for studying the cellular mechanisms underlying pressure-induced (myogenic) tone (4–6, 13, 14).

The appearance of tone in isolated cerebral arteries is highly dependent on the ambient level of transmural pressure. This observation has been reported in a number of earlier studies in which vessels are equilibrated at transmural pressures below the myogenic range, which is approximately between 40 and 140 mmHg for the rat posterior cerebral artery (17). As pressure is increased in a stepwise fashion, pressure-dependent tone develops spontaneously, most often between 40 and 60 mmHg. In this study, arteries were equilibrated at 60 mmHg, above the myogenic threshold pressure, and tone developed during equilibration. Studies were carried out on intact vessels because it is difficult to denude the endothelium in a highly branched preparation. Previous studies have shown that the endothelium modulates the level of tone but does not significantly alter myogenic responsiveness (12, 14).

From a mechanical standpoint, constriction results in VSM shortening and a considerable (>40%, 17) thickening of the arterial wall. Every isolated vessel develops its own level of MT, which, once present, can be maintained for hours with little subsequent change in diameter. What is not known, however, is how the level of tone is “set” in a particular vessel. Although circumferential tension provides the stimulus for tone development, it cannot be a set point for several reasons. First, tension is, by the Laplace definition, a product of radius and pressure. At a branch point where a small vessel emanates from a larger one (see Fig. 1), pressure would be similar, whereas radius may differ by severalfold (e.g., Fig. 4). Hence, wall tension in the smaller artery would be a fraction of that present in the larger trunk vessel. Second, tension does not account for the thickness of the vascular wall and especially of the force-producing component, i.e., VSM. Circumferential stress does take wall thickness into account and, in a pressurized cylinder, is defined as the force per unit area exerted by the wall (= VSM) in opposition to a distending force. To use the same analogy of a branch point, although pressure is similar in both vessels, and tension greater in the larger vessel (due to the greater radius), the presence of a thicker media containing several layers of VSM would also facilitate a reduction in force per unit area (stress). The hypothesis underlying this study is that the structure of an artery (specifically, cross-sectional area occupied by VSM cells) ultimately defines the degree of tone by normalizing stress at the contractile filament level.

These findings are also in agreement with the importance of circumferential tension as a stimulus (and as a regulated variable, e.g., 18, 20), given the caveat that it is not overall wall tension, but tension at the microfilament level, which is of primary importance. Stress merely normalizes tension based on cross-sectional area. From the the Laplace formulation, one would predict a gradient of increasing tension from the luminal to the abluminal side of the media (due to the increase in radius). On the other hand, some equaliza-
Fig. 5. Transmission electron microscopy image of posterior cerebral artery branches, fixed at 60 mmHg with stable myogenic tone, sectioned longitudinally to illustrate media thickness. A: small branch with single layer of vascular smooth muscle cells (VSMC), internal elastic lamina (IEL), and endothelial cell (EC). Diameter 35 μm, media thickness 4 μm. B: large branch with IEL, EC, and several VSMC layers. Diameter 205 μm, media thickness 14 μm.

Fig. 6. Relationship between intraluminal diameter and wall stress. A: wall stress, calculated from optical measurements of wall thickness, increased slightly as branch diameter increased. Linear regression line based on all values (n = 24 from 6 rats). B: comparison of wall stress in different-sized arterial branches, categorized as small and large (n = 8 for both). Data are plotted as means ± SE.

Fig. 7. Relationship between intraluminal diameter and media stress. A: media stress calculated from transmission electron microscopy images of longitudinally sectioned arterial walls expressed as a function of diameter. Linear regression line based on all values (n = 24 from 6 rats). B: comparison of media stress in different sized arterial branches, categorized as small and large (n = 8 for both). Data are plotted as means ± SE.
offers an approximation. Calculating media stress is a more accurate approach, because virtually all of VSM is normally contained within the tunica media. In small muscular arteries, the tunica media is extremely well organized and consists almost entirely of circumferentially arranged VSM (see Fig. 5).

As shown in Fig. 6, wall stress increases somewhat with increasing arterial size. Wall stress underestimates media stress. In pial arteries, the arterial wall seen through a light microscope includes the intimal and adventitial layers, and it is difficult to specifically measure media thickness, especially in small arteries. For this reason, we chose the indirect approach of fixing vessels once tone had developed and measuring media thickness directly from longitudinal sections of transmission electron micrographs. As shown in Fig. 7, media stress was not correlated with vessel size and was almost exactly the same in large versus smaller vessels.

If there is a normalization of stress at the VSM level in response to transmural pressure, one would predict that a similar degree of force is produced by individual VSM cells within large versus small arteries that possess tone. One way of evaluating this prediction is to measure media thickness and to calculate media stress, as already discussed. Another way is to measure a functional parameter directly related to force production, e.g., cytosolic calcium. Our results show that there was no difference in cytosolic calcium in different-sized vessels with tone (Fig. 3). Because all arterial branches experience an identical transmural pressure, this finding suggests that active cell stress (and force production) is equalized throughout the branching network (in the absence of size-dependent differences in Ca\(^{2+}\) sensitivity). Furthermore, the pial artery network is unique in that there is only a slight decrease in transmural pressure from the large (450 \(\mu\)m) to smaller (35 \(\mu\)m) arterial branches (19). Despite similar transmural pressure over a large range of artery size, there was still a difference in MT, whereas [Ca\(^{2+}\)], and media stress were approximately normalized.

The appeal of this concept of stress normalization lies in its potential for integrating arterial structure and function, at least in terms of arterial responses to intravascular pressure. One could argue that the number of VSM cells in a particular artery reflects the force-generating requirements of that vessel under normal conditions. Long-term pressure changes, such as hypertension, would lead to vascular wall adaptation by an increase in VSM mass (16, 17). In this regard, the association between resistance artery media hypertrophy and chronic hypertension is well documented and supports the constant stress hypothesis.

On the other hand, this concept also has its limitations. Clearly, arterial diameter in a functional vascular network is not invariable and can be modified by other influences, e.g., shear-stress induced alterations in endothelial production of vasoactive molecules, humoral vasoactive compounds, and neural activity (tissue metabolites may also play a role, although pial vessels are bathed in cerebrospinal fluid and relatively distant from neuronal cells). Changes in arterial caliber would, by definition, change wall and media stress and require some short-term adaptive responses. Second, significant fluctuations in systemic blood pressure occur secondary to physical activity, postural changes, and time of day. The level of cell stress would likely change as well, although the net effect may be to induce changes in arterial caliber associated with the process of cerebral blood flow autoregulation. An increase in pressure would result in constriction, which would, in turn, reduce the pressure-induced elevation in tension via the Laplace formulation (\(T = Pr\)) and reduce stress by the increase in wall thickness. Third, intravascular pressure is normally pulsatile, therefore media stress is not as discrete a quantity because it is under the static pressure conditions used in most in vitro studies, including ours. Nonetheless, it is intriguing to speculate that the resulting oscillation in stress may be a physiological signal in its own right, because several studies suggest that elevated pulse pressure, rather than mean pressure, is of primary importance in stimulating vascular remodeling in response to chronic hypertension (16).

In summary, the results of this study suggest that vessel-to-vessel differences in pressure-induced cerebral artery tone may be partly explained by an equalization of force production (and stress) at the smooth muscle cell level. In support of this, we found that cytosolic calcium concentrations in different-sized vessels with varying degrees of tone were approximately equal. Although wall stress, calculated in vessels with tone pressurized to 60 mmHg, increased with increasing arterial diameter, media stress (~VSM cell stress) was approximately equal in all arterial branches (~10% variation in spite of some 500% variation in arterial size).

The implication and significance of this finding is that vascular smooth muscle cell stress may represent a physiological set point that is relatively uniform and independent of resistance artery diameter. Regional differences in pressure-induced tone may therefore result from the equalization of force at the individual VSM level, thereby providing a mechanism for integrating arterial structure (e.g., lumen diameter, wall thickness, VSM area/unit length) with function (intrinsic tone).

The authors thank Keara McEllroy-Yaggy and Michelle VonTurkovich for assistance with vessel preparation and transmission electron microscopy.

The research in this study was made possible by the National Heart, Lung, and Blood Institute Grant HL-59406 (to G. Osol) and the Research Council of Norway (to J. F. Brekke).

REFERENCES


