Coordination of Mural Elements and Myofilaments During Arteriolar Constriction

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Arterioles undergo major morphological changes during vasoconstriction. We used transmission electron microscopy to study wall morphology in both dilated and constricted microvessels to understand the cellular basis of these changes. The relation between the orientation and density of myofilaments and the distribution of dense bodies was analyzed with respect to the level of microvessel tone. The data show a strong correlation between the degree of arteriolar constriction and both the orientation and density of myofilaments. In dilated arterioles, myofilament orientation was predominantly circumferential across the entire smooth muscle cell, averaging 84 ± 2° (SEM) relative to a radial reference line. In vessels constricted to 50% of their maximal diameter, myofilament orientation was dependent upon the location within the cell, being largely circumferential at the adventitial border (77 ± 4°) and shifting to a radial arrangement at the intimal border (36 ± 5°). The reorganization of myofilaments during constriction was associated with a decrease in myofilament density at the intimal-medial border of the smooth muscle cells. The decrease in myofilament density resulted from a selective withdrawal of myofilaments from periluminal areas where "ridges" had formed. Our observations suggest that an ordered distribution of membrane-associated dense bodies along the periluminal aspect of the smooth muscle cells is responsible for both the myofilament reorganization and ridge formation during vasoconstriction. Results of the present study are incorporated into a hypothetical model of arteriolar ultrastructure compatible with the mural reorganization observed during vasoconstriction. (Circulation Research 1986;59:620-627)

The purpose of the present study was to explore the relation between microvessel constriction, wall configuration, and smooth muscle cell ultrastructure. Our measurements use quantitative transmission electron microscopic analysis of hamster cheek pouch arterioles and focus on the smooth muscle force transduction elements; the myofilaments and membrane-associated dense bodies. In an attempt to understand the cellular mechanical forces acting on intact microvessels, we propose a model of arteriolar wall structure that integrates subcellular ultrastructure with the control of microvessel diameter.

Materials and Methods

Male golden hamsters (Charles River Supply) 103 ± 17 g (SEM) were anesthetized with sodium pentobarbital (70–80 mg/kg i.p.) and tracheostomized. Sodium pentobarbital was mixed with 154 mM NaCl (9.1 mg/ml) and infused continuously through a femoral vein cannula at a rate of 0.47 ml/hr to maintain acceptable levels of anesthesia and replace respiratory fluid loss. Deep esophageal temperature was monitored (Bailey Instruments #BAT-4) and maintained at 37–38° C by heat exchange coils placed under the animal.

The left cheek pouch was gently exposed and prepared for intravital observation in a manner previously described. The preparation was suffused with a physiological salt solution (PSS) containing (mM): NaCl, 131.9; KCl, 4.7; CaCl₂, 2.0; MgSO₄, 1.2; NaHCO₃, 20.0. This solution was bubbled with 5% CO₂ and 95% N₂ to yield a pH of 7.4 and maintained at 37° C. The preparation was transilluminated and observed with a Nikon Optiphot microscope equipped with a 55 X water immersion objective (NA 0.84).
Microvessels ranging in size from 30–91 μm in diameter when dilated with topical 10⁻⁴ M adenosine were examined. After constriction to preselected fractions of the dilated diameter (using topical 10⁻⁶ M norepinephrine), the arterioles were fixed in situ by immersing the entire cheek pouch in fixative (1.5% glutaraldehyde, 1.0% paraformaldehyde in PSS, 544 mOsm). Further processing for transmission electron microscopy utilized a modified version of a well established protocol for VSM cells and had no appreciable effect on vessel dimensions (< 5%). All subsequent processing was carried out at 4°C. The tissue was left in fixative overnight; small pieces (2 mm x 1 mm) of the pouch containing the vessels of interest were dissected out, rinsed in PSS for 30 minutes, and postfixed with 2% osmium tetroxide in PSS (246 mOsm) for 4 hours. The tissue cubes were rinsed in veronal acetate buffer for 30 minutes and stained en bloc with 3% uranyl acetate in veronal acetate buffer (215 mOsm) for 2 hours. The tissue was rinsed again in veronal acetate buffer, dehydrated in graded ethanol solutions (70–100%) at room temperature, and embedded in Polybed 812 (Polysciences). Thin cross sections (70 nm) of vessels were cut with a diamond knife (Diatome) on an LKB microtome (Ultratome III), and placed on formvar-coated slot grids. The sections were stained with uranyl acetate and/or lead citrate and examined at 60 kV on a Zeiss EM IOCA transmission electron microscope. Final micrograph magnifications ranged from 20,000–30,000 x.

Myofilament Orientation

Measurement of myofilament angle was performed by drawing a line tangent to the adventitial border of the smooth muscle cells and then constructing a line perpendicular to this tangent (Figure 1). This "radial" line was extended toward the lumen and used as a reference line for measuring myofilament angle. Three radial reference lines were constructed from randomly selected points along the circumference of each vessel. A simple radial line emanating from the center of the vessel was not used due to the bias introduced by vessels with elliptical rather than circular profiles.

The arteriolar media was divided into 10 equal increments along each radial reference line and myofilament angle measurements relative to this radial line were recorded at each increment point.

Myofilament Density

Myofilament density was measured to determine if distribution of the contractile machinery was uniform throughout the smooth muscle cell (perinuclear areas were excluded). Maximal myofilament density for each specimen was estimated by assessment of thick and thin filament number per unit area along the reference line on each vessel cross section. Once a maximal density had been obtained, myofilament densities across the muscle cells were normalized to the observed maximal density.

In selected graphs (Figure 3, bottom panel, and Figure 7), the area under the curve (measured using a planimeter, Microplan II, Cambridge, Mass.) of the periluminal region was divided by the total area under the curve to obtain fractional density and force generation values reported in the “Discussion” section.

Statistics

All statistical analyses utilized Student’s t test; differences were accepted as significant when p < 0.05.

Results

Constricted arterioles display a marked rearrangement of mural elements when compared with dilated controls (Figure 1). In constricted microvessels (Figures 1A, 1C) the endothelium, internal elastic lamina, and intimal aspects of the muscle cells form a series of prominent ridges. Furthermore, the amplitude of these intimar ridges increases with the degree of constriction. Compression of the endothelium into the lumen amplified the decrease in diameter produced by smooth muscle shortening.

Myofilaments

Because of the well-known variability in preservation of myosin filaments, measurements of myofilament orientation were made predominantly on thin filaments (6–8 nm), although thick filaments (13–15 nm) were also measured when present in the micrograph. To ensure that data collected from thin filaments also yielded a valid estimate of thick filament orientation, measurements were made of actin and myosin orientations in 40 randomly selected samples. Alterations in actin and myosin orientation paralleled each other across the entire cell as shown in Figure 2.

Myofilament orientation in dilated vessels was uniform across the entire smooth muscle cell and predominantly circumferential in arrangement ( • of top panel, Figure 3). In contrast, myofilament orientation in microvessels constricted to 50% of their maximal diameter was nonuniform ( • of top panel, Figure 3). A large change in constricted vessel myofilament angle was observed in the periluminal ridges (shaded region of top panel, Figure 3). In these vessels, myofilament arrangement within the constriction-induced ridges shifted to a radial configuration (mean angle = 36 ± 5°). This result was in marked contrast to the circumferential orientation of myofilaments (mean angle = 77 ± 4°) observed in the outermost (i.e., adventitial) aspects of the same cells.

A decrease in myofilament density also occurred in the periluminal ridges of constricted arterioles and followed a pattern of change similar to that observed for myofilament orientation (shaded region of bottom panel, Figure 3). Within the ridges of constricted vessels, myofilament density was found to decrease to approximately 20% of that observed in other areas of the cell.

The two panels of Figure 4 show the effects of increasing the level of constriction on myofilament orientation and density within the periluminal ridges. With increasing degrees of constriction, myofilament orientation becomes more radial while myofilament...
FIGURE 1. Transmission electron micrographs of hamster cheek pouch arterioles. A: Cross section of a 30 μm arteriole constricted approximately 50% with topical norepinephrine. Solid line delineates a tangent to the smooth muscle cell. Dashed line represents a radial line perpendicular to this tangent that was utilized as a reference line for measuring myofilament angle. Notice tortuosity of the endothelium (e), internal elastic lamina (iel), and vascular smooth muscle (vsm). rbc = red blood cell, adv = adventitia. B: Cross section of a 30-μm arteriole dilated with adenosine. Note circumferential arrangement of the mural elements. C: High magnification of area similar to that delineated by rectangular area shown in Figure 1A. Dashed line represents reference radial line for myofilament angle measurements. Smooth muscle cell ridges (rid) are apparent. Note areas labeled as base, side, and apex of a ridge; mf = myofilaments. D: High magnification of area similar to that delineated by rectangular area shown in Figure 1B. Note absence of ridges and endothelial folds. Scale bars = 1.0 μm.
density decreases. The change in myofilament orientation from a circumferential arrangement to a radial one was not observed in any region of the cell lying outside the periluminal ridges.

**Dense Bodies**

Dense bodies (DB) provide anchoring sites for thin filaments and permit the smooth muscle cell to transmit force to adjacent cells and the surrounding connective tissue. We assume that dense body distribution within the smooth muscle provides a marker for areas of mechanical coupling to the myofilaments. We therefore analyzed the distribution of dense bodies around the vessel circumference, both at the inner and outer borders of the arterioles. Our analysis shows a previously unreported pattern of dense body distribution in that portion of the smooth muscle cell bordering the intima (Figure 5, top panel). Dense bodies occur approximately twice as frequently at the base of a periluminal ridge as on either the side or apex. The underlying organization of the dense bodies becomes more apparent when one realizes that the base of a ridge is typically shorter than the combined lengths of the two sides. When normalized for the length of membrane involved, the incidence of dense bodies located on the bases is three times greater than that observed on either the apices or the sides of the ridge (Figure 5, bottom panel).

The incidence of dense bodies associated with the smooth muscle cell plasmalemma on the adventitial border was significantly elevated over that observed on the intimal border in both dilated and constricted microvessels (Table 1). The data also suggest that adventitial and intimal dense bodies change size and/or shape during constriction, typically becoming shorter and thicker. Values reported in Table 1 are means of the maximum width of dense bodies measured. Adventitial DB in constricted vessels frequently appeared wedge shaped and extended further into the cytoplasm than intimal DB.

**Discussion**

The present study demonstrates that arteriolar smooth muscle cell myofilaments undergo a reorientation during constriction. The reorientation of myofilaments and the decrease in myofilament density that occur periluminally are both functions of the degree of constriction within the microvessel. Our data suggest...
FIGURE 4. Relation of myofilament angle (top panel) and relative myofilament density (bottom panel) to the degree of arteriolar tone. Data obtained from the periluminal area (defined in this case only as the innermost 10% of the total smooth muscle cell width). Note decreases in both periluminal myofilament angle and density as the degree of constriction increases. n = 21 for 50 and 100% dilation, n = 3 for 25 and 75% dilations. Values = mean ± SEM.

that an ordered distribution of intimal dense bodies may play an important role in directing the formation of periluminal ridges created during vasoconstriction.

The conclusions and hypotheses which follow are based on the assumption that the electron microscopic techniques utilized in the present study accurately reflect the in vivo orientation of the myofilaments. We were particularly concerned with this aspect of the study since tissue processing protocols are known to alter myosin content as well as cellular volume and dimension. The concerns prompted us to develop a tissue-processing scheme for arterioles modified from a protocol used by others for cultured endothelial and smooth muscle cells.

We measured both thick and thin filament orientation and demonstrated a correlation between the two. This measurement assumes additional importance because some thin filaments form part of the structural (i.e., cytoskeletal) framework of the cell; are not directly involved in the contractile process, and hence their orientation may not accurately reflect vectors of force development.

Figure 6 is a scale drawing depicting the results presented in Figures 3–5 and illustrates changes that occur in the arteriolar wall during vasoconstriction. We propose it as a model of the interaction between subcellular morphology and wall structure in an intact microvessel. The pivotal element of the model is the periodic placement of dense bodies along the intimal-medial border of the vessel wall. We assume, though it remains to be demonstrated experimentally in these particular smooth muscle cells, that the dense bodies are arranged longitudinally in rows along the vessel axis and occur preferentially in locations which ultimately become the bases of periluminal ridges during constriction. It is interesting to note that if this is the case, the narrow width (3–5 μm) of hamster vascular smooth muscle cells requires that dense bodies be arranged in register between adjacent cells.

Given this organization, it can be anticipated that arteriolar vasoconstriction will force the endothelial cells as well as that portion of the smooth muscle between rows of dense bodies into the lumen of the vessel. The result is creation of ridges that extend for several hundred microns along the length of the vessel. An additional outcome of this coordinated mo-

FIGURE 5. Top panel: Membrane-associated dense body (MADB) frequency (%) along the intimal border of constricted smooth muscle cells. MADB frequency at the base of a ridge (see insert) was significantly elevated over that observed in other areas. Insert is a schematic representation of periluminal ridges in constricted arterioles illustrating the different portions of a ridge. Bottom panel: Fraction of periluminal smooth muscle cell membrane occupied by MADB in constricted arterioles. The incidence of dense bodies at the base of a ridge was approximately 3 times that observed on the sides or apex. Values = mean ± SEM, n = 8 vessels. * = p < 0.05.
tion of the wall elements is to amplify the luminal reduction induced by a given amount of smooth muscle shortening.16

Figure 6 suggests an explanation for many of the changes in cell shape and myofilament orientation observed in the present study. We envision myofilaments spanning the smooth muscle cell from an adventitial surface dense body to an intimal dense body (presumably via intervening cytoplasmic dense bodies). The marked change in filament orientation within the ridges is a consequence of their passive rotation as the sides of a ridge approach each other during constriction. Withdrawal of filaments from these corrugations during shortening may be understood if a large portion of the force-transducing filaments are attached to dense bodies located at the bases of ridges. The ridges can be regarded as extrusions of the smooth muscle cell cytoplasm toward the lumen in the space between rows of dense bodies. We believe the ridges observed in the periluminal areas of constricted microvessels to be analogous to, but distinct from, the cellular evaginations observed by Fay and Delise4 in constricted isolated smooth muscle cells.

Our data are compatible with the observations of Gabella17 and Fay et al18 showing a highly organized pattern of dense body distribution within smooth muscle cells. There is, however, both a size and orientation difference between the constriction-induced alterations we report and those observed by others in isolated smooth muscle cells.4 The structures observed in isolated cells are evaginations approximately 0.1–0.8 μm in diameter while the ridges we observe in constricted arterioles range from 0.75–2.1 μm in width. In addition, the dense bodies observed in isolated cell studies are arranged in longitudinal arrays with respect to the long axis of the cell.18 This arrangement is in contrast to our results where dense bodies exist in arrays that are organized with respect to the transverse axis of the cell and are associated with the bases of constricted arteriolar ridges.

Although the common view is that the dense bodies act as the equivalent of skeletal muscle Z-lines,19–21 this idea is not universally accepted.22,23 We ascribe to the view that dense bodies are the key attachment point for myofilaments in smooth muscle, but this idea must be viewed with some caution since our observations indicate that dense bodies appear to shorten and thicken during constriction (Table 1).

The limitations of using electron micrographic cross sections to analyze complex structural geometry are well known.24 The apparent change in dense body dimensions reported in Table 1 could in fact result from a change in dense body shape or orientation. Thus the data, while intriguing, cannot be considered a definitive report of dense body ultrastructure. It is possible that only part of the dense body is the true anchor point for contractile filaments and that the remainder collapses passively during shortening. It is also possible that there are some other cytoskeletal filament attachments that contribute to the observed dense body deformation. Such attachments have been reported.25 Further studies are required to evaluate the relations among myofilaments, dense bodies, and the smooth muscle cell cytoskeleton.

Our observations lead us to conclude that the formation of periluminal corrugations during vasoconstriction is not a random or passive phenomenon but results from a highly organized smooth muscle cell ultrastructure. Since cell membranes are incapable of undergoing significant elastic recoil, reductions in vessel diameter are associated with folding of the “excess” membrane. If a vessel is to function effectively in the control of blood flow, it must maintain a circular luminal cross section.26 We suggest that the anatomic changes observed in the present study provide a basis

<table>
<thead>
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<th>DB location</th>
<th>Vessel condition</th>
<th>DB circumferential length (μm)</th>
<th>DB radial width (μm)</th>
<th>DB length/membrane length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal</td>
<td>Dilated</td>
<td>2.1 ± 0.2*†</td>
<td>0.3 ± 0.04*†</td>
<td>0.16 ± 0.2*†</td>
</tr>
<tr>
<td></td>
<td>Constricted</td>
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<td>0.5 ± 0.05*</td>
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</tr>
<tr>
<td>Adventitial</td>
<td>Dilated</td>
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<td>0.5 ± 0.1†</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Constricted</td>
<td>1.7 ± 0.1</td>
<td>2.1 ± 0.07</td>
<td>0.28 ± 0.09</td>
</tr>
</tbody>
</table>

Values = mean ± SEM. n = 8 vessels for each location. *p < 0.05 when compared to adventitial values. † = p < 0.05 when compared to constricted values from the same location.

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**Table 1. Membrane-Associated Dense Body (DB) Measurements**

**FIGURE 6.** Schematic drawing of a portion of an arteriolar wall detailing mural element reorganization in dilated and constricted states. See Discussion.
for the regular series of ridges observed along the intimal border of the vascular smooth muscle cells and allow the lumen of an arteriole to maintain an approximately circular profile during vasoconstriction.

In an analysis of force distribution within the arteriolar wall, Greensmith and Duling proposed that the effective cross-sectional area for force generation should be considered as only that portion of the media outside the ridges, the so-called "apparent effective fraction." The data presented in Figure 3 support this assertion. Both the reorientation and the withdrawal of filaments from the ridges contribute to a reduction in the force-generating ability of the smooth muscle within the ridges. The combined effects of the two processes are estimated (Figure 7) by computing the product of the fractional tangential force vector (sine of the filament angle) and the relative filament density. This calculation indicates that the net force generated by myofilaments within the ridges is quite small. Although 7.5% of the myofilaments are present in the ridges, they generate only 4.8% of the total smooth muscle cell force production.

We have analyzed two intracellular structures (i.e., myofilaments and dense bodies) responsible for changes in smooth muscle cell morphology during constriction. It is also likely that intercellular structures serve to coordinate the alterations in cellular morphology between adjacent cells. One such structure that could coordinate creation of periluminal ridges is the myoendothelial junction. We suggest that myoendothelial junctions could play a key role in coordination of cell movements in the constricting arteriolar wall. Further investigation of the frequency and distribution of myoendothelial junctions is required to resolve their possible role in ridge formation during constriction.

In conclusion, we have endeavored to correlate arteriolar ultrastructural changes with the functional state of smooth muscle cells during vasoconstriction. Our results complement previous observations obtained from cheek pouch arterioles of comparable size. We present new data pertaining to the creation of periluminal ridges and force generation within the arteriolar media. A decrease in myofilament density and a change in myofilament orientation in the periluminal region of constricted microvessels are the principal findings of this study. Such a reorganization of myofilaments maximizes tangential force-generating ability of an arteriole and supports speculation that force generation is reduced in the periluminal ridges of constricted vessels. Finally, a periluminal pattern of dense body distribution was observed that may direct creation of periluminal ridges in constricted microvessels and account for the alterations of myofilament orientation and density observed in the present study.

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References


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