# Factors Influencing the Expression of Stress Fibers in Vascular Endothelial Cells In Situ

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ABSTRACT The organization of actin and myosin in vascular endothelial cells *in situ* was studied by immunofluorescence microscopy. Examination of perfusion-fixed, whole mounts of normal mouse and rat descending thoracic aorta revealed the presence of axially oriented stress fibers containing both actin and myosin within the endothelial cells. In both species, the proportion of cells containing stress fibers varied from region to region within the same vessel. Some endothelial cells in mouse mesenteric vein and in rat inferior vena cava also contained stress fibers. Quantitative studies of the proportion of endothelial cells containing stress fibers in the descending thoracic aorta of age-matched normotensive and spontaneously hypertensive rats revealed significant differences. When animals of the same sex of the two strains were compared, the proportion was approximately two times greater in the spontaneously hypertensive rats. The proportion of endothelial cells containing stress fibers was about two times greater in males than in females of both strains. These observations suggest that multiple factors, including anatomical, sex, and hemodynamic differences, influence the organization of the endothelial cell cytoskeleton in situ.

Stress fibers in tissue-cultured cells are linear, phase-dense, cytoplasmic fibrils that are demonstrable by light microscopy and consist of bundles of microfilaments (3). Immunofluorescence techniques have revealed actin and myosin, as well as accessory contractile proteins, within these structures (15, 24, 25, 40), but their functional significance in vitro has remained unclear (4). Furthermore, on only a few occasions have similar structures been observed in situ (6, 32, 42–44).

Using immunofluorescence microscopy, work in this laboratory (6) demonstrated stress fibers in the scleroblasts located near the edge or over the radial ridge of the fish scale. Inasmuch as these regions are likely to experience great shearing forces, this suggested that in situ stress fibers play a role in cellular adhesion. Stress fiber-like structures were also observed within other cells but their function was interpreted as being different from that of the fibers found in the scleroblasts. As early as 1953, Palade (29) observed filaments within the basal cytoplasm of capillary endothelial cells. Subsequently, many investigators, using transmission electron microscopy, reported the presence of microfilaments (presumably containing actin; see reference 11) in the endothelial cells of various blood vessels in normotensive animals (20). In certain endothelial cells, these microfilaments were organized into bundles (7). Cross-striated microfilament bundles (similar in appearance to the stress fibers of certain tissue-cultured cells) have been observed in the arterial endothelium (aorta and cerebral arteries) of hypertensive rats (17, 19). However, similar structures were not found in normotensive rats; thus, it was postulated that cross-striated microfilament bundles are an adaptive response of the endothelial cell to hypertension (17). Similar cross-striated microfilament bundles were found in certain fibroblasts ("myofibroblasts") present in healing wounds (18). On the basis of their structural resemblance to myofibrils, these stress fiber-like structures were postulated to be contractile (for a review, see reference 20).

We chose vascular endothelium as a model system for investigating the functional significance of stress fibers in situ. Not only is there a large body of published reports on endothelial cell biology, but this tissue offers unique advantages for such a study. For example, hemodynamic forces might be expected to influence the cytoskeleton of the endothelial cell, inasmuch as such forces have been shown to influence endothelial cell shape both in vivo and in vitro (9, 33, 37). Furthermore, the availability of spontaneously hypertensive rats (28) permits the study of endothelial cells in an animal model in which both the genetic and hemodynamic factors are relatively well defined (13, 30, 39, 45).

The present study was undertaken to determine whether



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only the arterial endothelial cells of hypertensive animals contain stress fibers and whether there are factors that influence the expression of this structure within the endothelial cell. The aorta and other large blood vessels can be fixed by perfusion in situ, and their endothelial lining can be examined as a whole-mount preparation. By this approach, large numbers of endothelial cells can be examined in their native position. Using immunofluorescence microscopy with antibodies to actin and to myosin, we demonstrated the presence of stress fibers within the endothelial cells of normal mouse and rat thoracic aorta. Furthermore, we noted clear differences between the cytoskeletal organization of arterial endothelial cells in normotensive and spontaneously hypertensive rats. Our observations suggest that the expression of stress fibers in the vascular endothelium is influenced by multiple factors, including anatomical location, sex, genetic make-up, and hemodynamic forces.

## MATERIALS AND METHODS

*Animals:* Normal adult male and female CD-1 mice weighing 35–45 g were purchased from Charles River Breeding Laboratories (Wilmington, MA). Normal adult female Sprague-Dawley rats weighing 125–140 g were obtained from the same source. Eight-week-old male and female Wistar-Kyoto rats (normotensive control strain) and spontaneously hypertensive rats (Okamoto-Aoki type [28]) weighing 140–150 g were purchased from Taconic Farms (Germantown, NY). The females of both strains were routinely ovariectomized by the supplier and were used within 1 wk of the surgery. All animals were maintained on a diet of Purina rat laboratory chow (Ralston Purina Co., St. Louis, MO) and tap water *ad libitum*.

*Perfusion Fixation:* The perfusion fixation procedure of Forssmann et al. (12) was followed. The animals were anesthesized by an intraperitoneal injection of either tribromoethanol (0.2 ml of a 2.1% solution/10 g of body weight) or chloral hydrate (0.5 ml of a 7.4% solution/100 g of body weight), the abdominal aorta was cannulated, and the inferior vena cava (infrarenal or hepatic region) was cut. Following a brief (5 s) perfusion with "rinse solution" (0.9% NaCl, 2.5% polyvinylpyrrolidine, $M_r$ 40,000 [Sigma Chemical Co. St. Louis, MO], 0.025% heparin [Sigma Chemical Co.], 0.5% procaine-HCl [Sigma Chemical Co.], pH 7.4), 100–200 ml of a fixative mixture consisting of 2% formaldehyde, 0.1% picric acid, 50 mM sodium cacodylate (pH 7.4) was introduced. All animals were perfused at 90 mm Hg hydrostatic pressure for 15 min to ensure controlled distension of the blood vessel wall during fixation. Exclusion of the rinse solution made perfusion more difficult, and the quality of the immunofluorescent image was poor, but stress fibers nevertheless were clearly visible. Both anesthetics yielded similar fixation quality and cytoskeletal staining patterns. However, as will be discussed in Results, the concentration of formaldehyde significantly influenced the cytoskeletal staining patterns obtained. After the perfusion, the descending thoracic aorta (from the lower arch to the diaphragm) was excised, pinned down on dental wax, and flooded with phosphate-buffered saline (0.85% NaCl, 10 mM sodium phosphate, pH 7.4 [PBS]). The adventitia was carefully removed, and the vessel was cut open lengthwise between the intercostal arteries (i.e., along its dorsal aspect). The original direction of blood flow was noted. The vessel was cut crosswise into multiple segments 2–3 mm in width, and each segment was processed for immunofluorescence microscopy as described below. No attempt was made to strip off the intimal lining or to otherwise make an endothelial "Häutchen" preparation (34). The mesenteric vein and inferior vena cava (infrarenal portion) were handled in a similar manner. Fig. 1 illustrates some of these procedures.

*Antibodies:* Antibodies were raised in rabbits and characterized as previously described. The anti-myosin antibody is directed against human platelet myosin (15). This antibody has also been conjugated to tetramethylrhodamine and was used in the double-label experiment. The anti-actin antibody is directed against fish skeletal muscle actin and was affinity purified (6). The antitubulin antibody is directed against vinblastine-induced tubulin crystals from sea urchin eggs (16). Rhodamine-labeled goat anti-rabbit IgG was purchased from Miles-Yeda, Rehovot, Israel (lot 17179), and fluorescein-labeled sheep anti-rabbit IgG was purchased from Wellcome Reagents, Ltd., Beckerham, England (lot K5592).

*Immunofluorescence Procedures:* After dissection, the fixed aortic segments were washed three times with PBS (5 min per wash), permeabilized by treatment with −20°C acetone for 5 min, and washed three times with PBS (1 min per wash) to remove the acetone. The segments then were incubated at

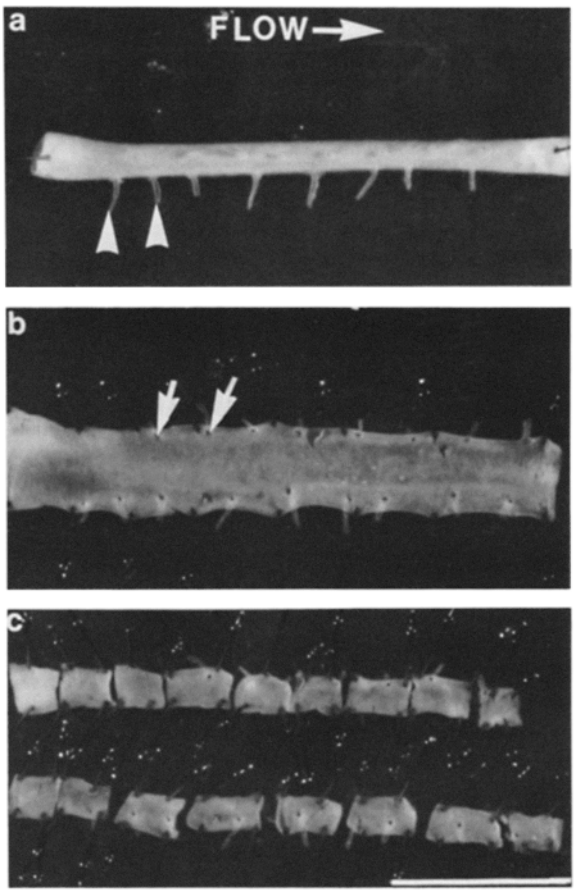


FIGURE 1 Preparation of rat thoracic aorta segments. (a) After in situ perfusion fixation, the aorta was excised and pinned out on dental wax with the direction of blood flow indicated. Several intercostal arteries can be seen (arrowheads). (b) The adventitia was carefully dissected away, and the vessel was incised along its dorsal aspect between the paired openings (arrows) of the intercostal arteries and pinned down. (c) Serial segments, 2–3 mm wide, were cut and prepared for immunofluorescence microscopy, and their original axial position and flow orientation were noted. Bar, 10 mm.

37°C with 50 μl/segment of affinity-purified anti-actin (50 μg/ml) or antimyosin (anti-serum diluted 100 times in PBS) in a moist atmosphere for 30–45 min. After three 5-min washings with PBS, the labeled secondary antibody (diluted 100 times in PBS) was applied, and a comparable incubation (37°C for 30–45 min) was performed in darkness. For the double-label experiment, the first incubation was with anti-actin, the second with fluorescein-labeled sheep anti-rabbit IgG, and the third (37°C for 30–45 min) with rhodamine-labeled anti-myosin (120 μg/ml; three dyes/IgG). After three 5-min washings in PBS, the aortic segments were mounted in 90% glycerol in PBS. For each segment, its axial position and its orientation with respect to the in vivo direction of blood flow were noted.

Several control stainings were carried out to establish the specificity of the endothelial cell staining patterns. For anti-myosin, preimmune serum and antigen-absorbed immune serum were used as the primary antibody instead of the specific antibody. In the case of anti-actin, the antigen-absorbed immune IgG preparation was tested. In these controls, there was only a faint general fluorescence over the entire specimen. The vessel segments also were tested for autofluorescence as well as for the nonspecific binding of the fluorescein-labeled sheep anti-rabbit and the rhodamine-labeled goat anti-rabbit antibodies. Low-intensity autofluorescence, as well as some nonspecific binding of the labeled secondary antibodies, was noted; however, these did not interfere with the visualization of the specific staining patterns. Staining the endothelium with an anti-smooth muscle myosin antibody (31) that does not cross-react with endothelial myosin (23) gave only a faint fluorescence in the plane of the endothelium.

Fluorescence microscopy was performed with a Leitz Orthoplan microscope equipped with a Ploemopak 2 illuminator with a Leitz L-2 filter block, and a

Zeiss 63 × Planapo phase-contrast objective lens (NA 1.4, oil). Fluorescent images were photographed with a Leitz Orthomat automatic camera with Tri-X film (Eastman Kodak Co., Rochester, NY). The film was exposed at ASA 1000 and developed with Acufine developer (Acufine, Inc., Chicago, IL). A scale with 10-μm divisions was photographed at the same primary magnification to calibrate the micrographs.

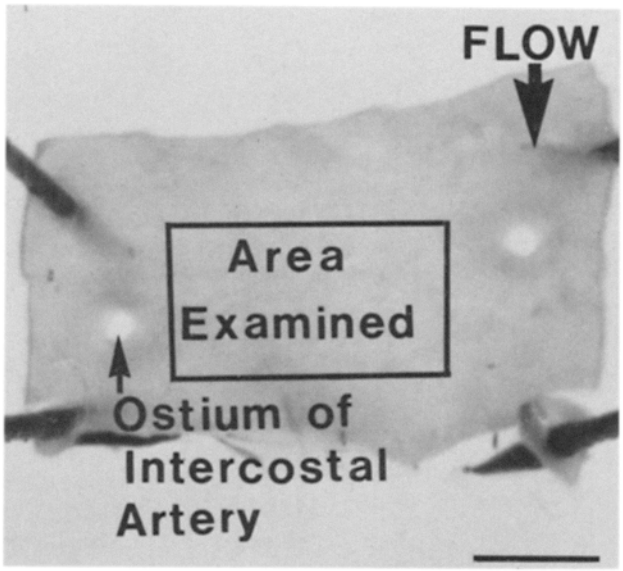


FIGURE 2 Region of descending thoracic aorta examined. The superimposed rectangle indicates the region of the ventral aortic wall in which the majority of the microscopic observations were made. For quantitative, comparative studies in normotensive and hypertensive rats, six randomly selected areas within the rectangle were examined. Bar, 1 mm.

*Quantitative Methods:* For the quantitative study comparing the proportion of aortic endothelial cells containing stress fibers, 12 Wistar-Kyoto rats (6 males, 6 females), and 12 spontaneously hypertensive rats (6 males, 6 females) were used. In each animal, 1,000–1,500 endothelial cells were examined in six randomly selected, 150 × 250-μm areas located along the ventral aspect of the thoracic aorta at the levels of the ostia of the first through sixth intercostal arteries (Fig. 2). All counts were made directly on the epifluorescence microscope and were verified independently by a second observer. Length (>4 μm) and the appearance of striations with the antimyosin antibody were the two main criteria used to identify a stress fiber.

## RESULTS

### *Actin and Myosin Organization in Vascular Endothelium In Situ*

GENERAL EXPERIMENTAL APPROACH: Fig. 1 illustrates the basic features of the dissection procedure used to prepare segments of mouse and rat thoracic aorta and other blood vessels for immunofluorescence microscopy. After in situ perfusion fixation, the dissected segments were examined by epifluorescence illumination as "whole mounts." This preserved the original anatomical relationships of the endothelial lining. Examination by phase-contrast microscopy revealed that broad areas of the intima remained intact in these preparations. Approximately 250 vessel segments were examined from 13 male and female CD-1 mice, 3 female Sprague-Dawley rats, and 24 male and female Wistar-Kyoto and spontaneously hypertensive rats.

MOUSE THORACIC AORTA: The immunofluorescent pattern produced by the staining of the descending thoracic aorta of CD-1 mice with the anti-actin antibody consisted of cortical staining, which delineated the cell shape, and prominent fibrillar staining within the cytoplasm (Fig. 3*a*). The

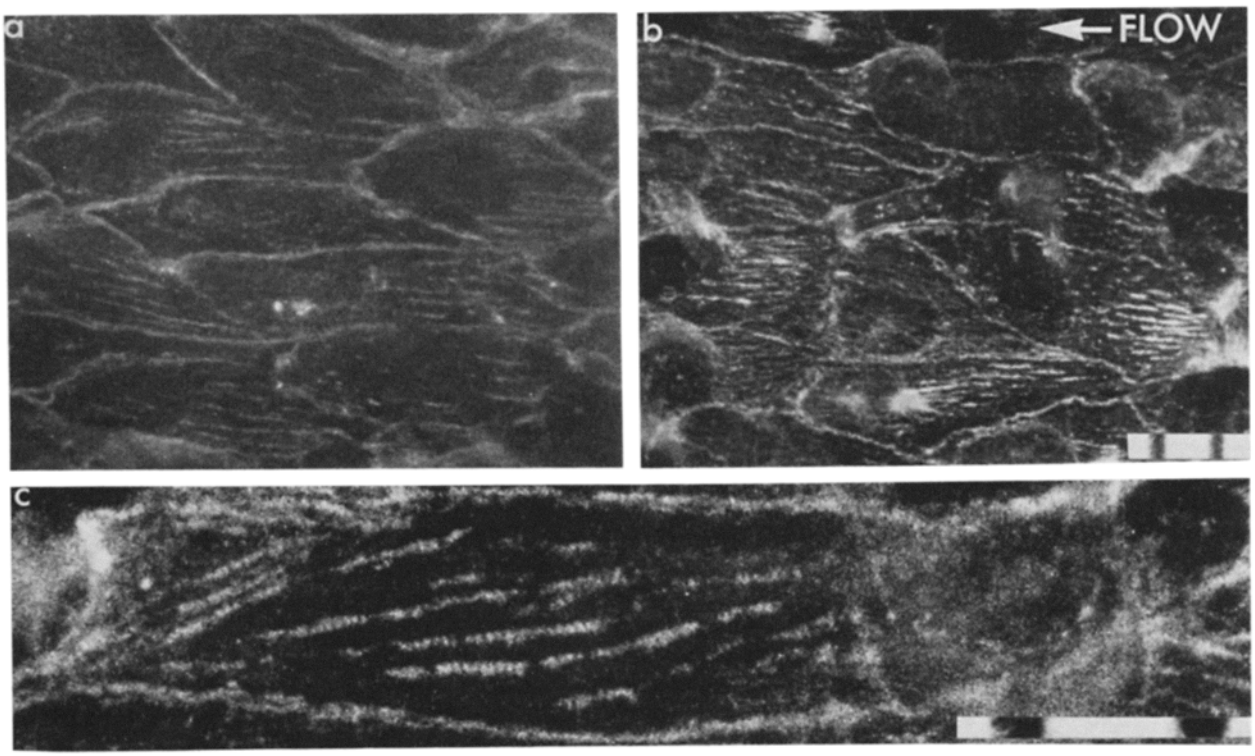


FIGURE 3 Indirect immunofluorescent localization of actin and myosin in the endothelial lining of mouse thoracic aorta. The arrow indicates the direction of blood flow in vivo. (*a*) Anti-actin staining pattern. Note the localization of fibers in the proximal end of each cell. In this particular field, the majority of the cells have actin-containing cytoplasmic fibers. (*b*) Anti-myosin staining pattern. (*c*) Enlargement of a single cell stained with anti-myosin to demonstrate the striated pattern along individual cytoplasmic fibers. The periodicity of the striations (measured from the center of the bands) is approximately 0.5 μm. Micrometer division, 10 μm.

cells examined were located in the center of the aortic segments (a similar area is indicated in Fig. 2), and they typically were ellipsoid, with their long dimension parallel to the vessel axis. The cytoplasmic fibrillar staining pattern showed several interesting features. First, individual actin-containing fibers were of uniform width (~0.7 μm) but varied in length from 4 to 25 μm. Second, they were aligned with the major axis of the cell, and, thus, appeared to be oriented parallel to the macroscopic direction of blood flow. Third, the majority of these fibers were located in the "proximal" end (in relation to the blood flow) of the cell and usually did not extend downstream beyond the nucleus. Fourth, the number of fibers observed within a given cell varied but rarely was greater than 10. Finally, the proportion of cells containing these fibers (defined as the ratio of endothelial cells containing such fibers to the total number of endothelial cells examined) varied from region to region along the descending thoracic aorta. In some areas, this ratio was as high as 0.4.

There were no discernible qualitative differences in the anti-actin staining patterns of male and female mice. For this species, no attempt was made to determine whether there were any quantitative differences between the sexes in the expression of these fibers. As indicated in Materials and Methods, the pattern of anti-actin staining, however, was significantly influenced by fixation conditions. Concentrations of formaldehyde greater than 2% (2, 3.7, 4, and 6% solutions were tested) gave more punctate cytoplasmic staining, with a marked reduction in the number of cells containing actin fibers; the staining intensity, however, was the same for all concentrations. The picric acid, while not essential, did improve the contrast of the image slightly but had no effect on the number of stress fibers visualized.

Anti-myosin staining of mouse aortic endothelium also showed both a cortical and a cytoplasmic fibrillar component (Fig. 3*b*). As seen with anti-actin, myosin-containing cytoplasmic fibers were most prominent in the proximal portion of the cells and had a definite axial alignment with the direction of blood flow. These structures exhibited a banded or striated staining pattern (Fig. 3*c*) similar to that seen in the stress fibers of tissue-cultured cells. The periodicity of these striations, as measured from the center of the bands, is ~0.5 μm, which is slightly less than that found in tissue-cultured cells. Consistent with the anti-actin results, not every cell exhibited a cytoplasmic fibrillar staining pattern with anti-myosin. However, this reagent did produce a more diffuse cytoplasmic staining than anti-actin, permitting visualization of axially oriented, ovoid endothelial cell nuclei. Inasmuch as fibers were not found in every cell examined, this staining characteristic was useful in documenting the presence of individual cells in which cytoplasmic fibers were not visible. Directly staining this tissue with rhodamine-labeled anti-myosin produced identical results, although the fluorescence intensity was lower.

To determine whether the anti-actin and anti-myosin fibrillar staining represented the same or different fiber systems, a double-label experiment was performed. If the actin and myosin colocalized in the same fibers, then these structures might more accurately be referred to as "in situ stress fibers." When the same tissue segment was stained indirectly for actin and then directly for myosin (see Materials and Methods for details), both antibodies stained the same fibrillar structures (Fig. 4), providing further morphological evidence that these structures are stress fibers. Inherent in this experimental design is the possibility that the fluorescein-labeled sheep anti-rabbit IgG used for the indirect labeling of actin could uncouple from the anti-actin antibody and bind to the rhodamine-labeled anti-myosin antibody, giving a spurious colocalization pattern. This potential problem was tested for by staining cultured bovine aortic endothelial cells indirectly for tubulin and directly for myosin by a similar experimental method. The two cytoskeletal patterns (microtubules and stress fibers) were not superimposable, showing that our experimental conditions do not result in spurious colocalization of the two antigens.

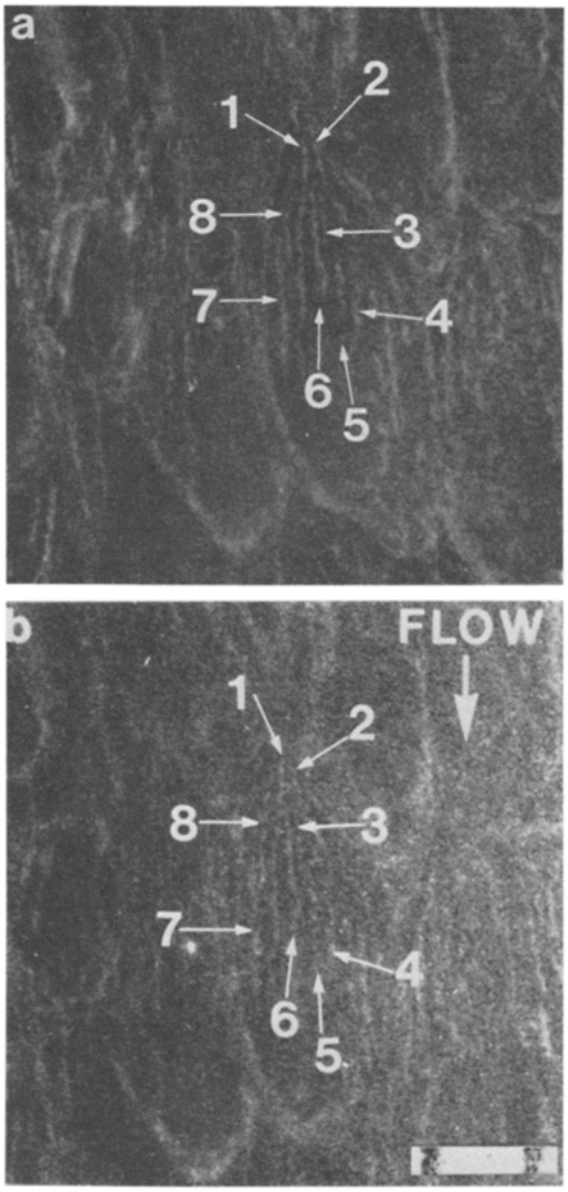


FIGURE 4 Colocalization of actin and myosin in stress fibers in endothelial cells of the mouse thoracic aorta. (*a*) Anti-actin staining. The numbered arrows indicate eight stress fibers within this cell. (*b*) Anti-myosin staining pattern of the same microscopic field as in *a*. The eight stress fibers identified in *a* can be found here, indicating that both antigens are located in the same structures. Micrometer division, 10 μm.

MOUSE MESENTERIC VEIN: Because the endothelial lining of the thoracic aorta is subjected to relatively high levels of shear stress, a relatively low shear stress vessel such as the

mesenteric vein also was studied. Mouse mesenteric vein endothelial cells had more rounded nuclei and exhibited a range of cell shapes, varying from ellipsoid to polygonal. Furthermore, their immunofluorescent anti-myosin staining pattern revealed a stress fiber organization different from that observed in the thoracic aorta. Occasional stress fibers were discernible, but there was no apparent orientation of cytoskeletal elements with the direction of blood flow.

SPRAGUE-DAWLEY RAT THORACIC AORTA AND INFERIOR VENA CAVA: In contrast to that of the mouse, female Sprague-Dawley rat vascular endothelium showed qualitatively similar anti-myosin staining patterns in the descending thoracic aorta and the inferior vena cava. Unfortunately, in all strains of rat examined in this study, the anti-actin antibody produced only a faint staining pattern. Stress fibers were clearly discernible by microscopy, but the low-intensity staining made photography difficult. In the descending thoracic aorta, anti-myosin again showed cortical staining as well as a striated staining pattern along the stress fibers. In contrast to mouse, the proportion of endothelial cells containing stress fibers within this vessel was low, and rarely exceeded 0.1. In the inferior vena cava (infrarenal region), endothelial cells again were variously shaped and had more rounded nuclei. In contrast to the endothelial cells of the mouse mesenteric vein, however, certain of these endothelial cells appeared to contain occasional axially oriented stress fibers.

## *Normotensive vs. Spontaneously Hypertensive Rats*

COMPARISON OF PROPORTION OF ENDOTHELIAL CELLS CONTAINING STRESS FIBERS: The studies presented thus far demonstrate that stress fibers are present in normal mice and rats. They also reveal that stress fibers are encountered much more frequently in arterial than in venous endothelium, which suggests that blood pressure or, more generally, hemodynamic factors influence the expression of stress fibers in endothelial cells in vivo. To investigate whether hemodynamic factors affect the extent of stress fiber development in endothelial cells, comparative studies were performed with Wistar-Kyoto (normotensive) and spontaneously hypertensive rats, strains that are genetically related but whose blood pressures (28) are different.

The proportion of endothelial cells containing stress fibers in the descending thoracic aorta was determined in male and female Wistar-Kyoto and spontaneously hypertensive rats at 8 wk of age. The data were gathered from cells lining the ventral aspect of the aorta (away from the orifices of the intercostal arteries), where the blood flow could be expected to be laminar (Fig. 2). There were marked strain- and sex-related differences in endothelial cell stress fiber expression (Table I). Spontaneously hypertensive rats of both sexes showed a greater than twofold higher proportion of endothelial cells containing stress fibers than sex-matched, normotensive Wistar-Kyoto rats. In addition, within each strain, the proportion of endothelial cells containing stress fibers was significantly greater in males than in females. Statistical treatment of the data (Student's $t$ test) revealed that the differences between the two strains within the same sex and the differences between the sexes within the same strain are significant ($P < 0.01$). The number of stress fibers per cell was relatively low (averaging five or fewer per cell) in both strains; in both the Wistar-Kyoto and the spontaneously hypertensive rats, however, aortic endothelial cells with 6–10 stress fibers occasionally were found.

In addition to these quantitative differences, certain qualitative differences between the two strains were apparent. In normotensive Wistar-Kyoto rats, the stress fibers tended to have a thinner or more wispy appearance, but they retained a cross-striated pattern. In spontaneously hypertensive rats, the majority of the stress fibers appeared thicker and had more prominent striations (Fig. 5). In addition, aortic endo-

TABLE I

*Expression of Stress Fibers in Rat Aortic Endothelial Cells*

| Strain* | Proportion of Cells Containing Stress Fibers‡§ | |
|---|---|---|
| | Male | Female |
| Wistar-Kyoto (normotensive) | 0.188 ± 0.009 | 0.098 ± 0.007 |
| Spontaneously hypertensive | 0.460 ± 0.013 | 0.217 ± 0.009 |

* 12 animals of each strain (6 males, 6 females); 8 wk old

‡ Proportion = number of endothelial cells containing stress fibers/total number of endothelial cells examined (see text for details)

§ Data expressed as proportion ± 2 SD

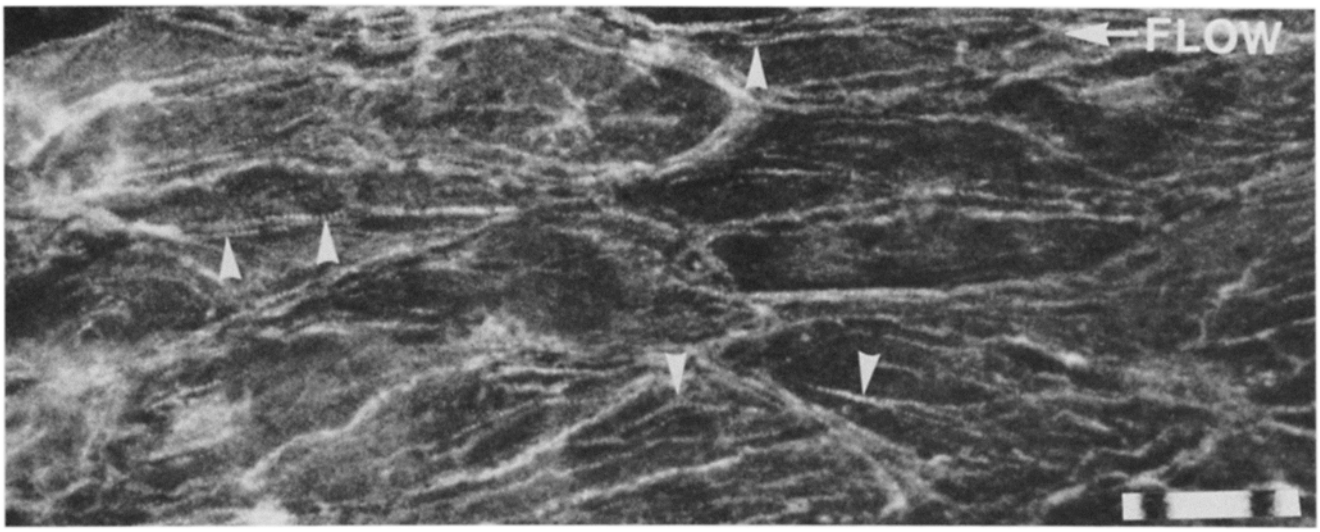


FIGURE 5 Anti-myosin staining pattern of the aortic endothelium in a male spontaneously hypertensive rat. Note the prominent stress fibers (arrowheads) in which striations are clearly visible. Micrometer division, 10 μm.

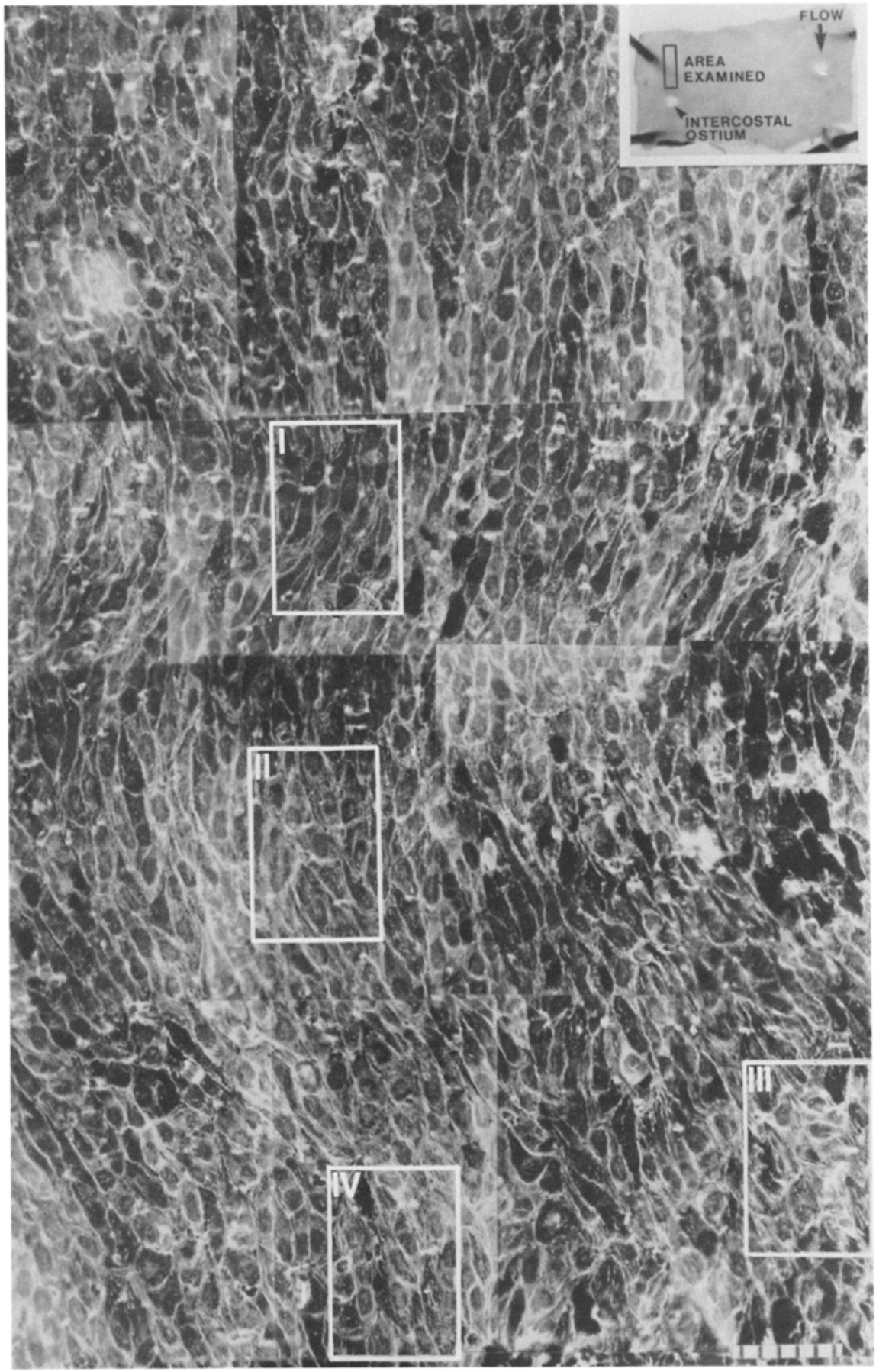


FIGURE 6 Photographic reduction of a montage of the anti-myosin staining pattern at the level of the sixth intercostal artery of a male spontaneously hypertensive rat showing regional variation in stress fiber expression. The *inset* indicates the location of the area shown. Region I: very few, if any, cells contain stress fibers. Region II: almost all of the cells contain prominent stress fibers. Region III: endothelial cell shape is variable and not all stress fibers are aligned with the long axis of the vessel (see enlargement of this region in Fig. 7). Region IV: almost all of the cells have prominent stress fibers. Micrometer division, 10 $\mu$m.

thelial cells in the spontaneously hypertensive rats tended to be more ellipsoid than their Wistar-Kyoto counterparts.

REGIONAL VARIATION IN THE EXPRESSION OF STRESS FIBERS: As the data summarized in Table I indicate, not every aortic endothelial cell examined contained stress fibers. In male spontaneously hypertensive rats, in which approximately one-half of the cells contained stress fibers, no clearly discernible pattern of "stress fiber-positive" cells within the region of the vessel examined (Fig. 2) was observed. Within the descending thoracic aorta there was a regional variation in the distribution of stress fiber-positive cells. Fig. 6 is a montage of a 0.48 × 0.79-mm area of anti-myosin-stained aortic endothelium from a male spontaneously hypertensive rat at the level of the sixth intercostal artery. Although this montage was not taken from the area from which the data for determining the proportions were gathered (see Fig. 2), it illustrates the regional variation found throughout the descending thoracic aorta in all animals examined. Four areas from Fig. 6 were selected to illustrate the regional variation of stress fiber expression. In region I, almost every cell was stress fiber-negative. In region II, almost every cell was stress fiber-positive. In region III, several cells in the middle of the field were not ellipsoid and contained stress fibers oriented perpendicular to the direction of blood flow within the vessel (see Fig. 7). In region IV, again, almost all cells are stress fiber-positive. Note that in regions I, II, and IV, the cells are similarly shaped, yet not all have stress fibers. As seen in the inset of Fig. 6, the lower portion of the area displayed in the montage was just proximal to the ostium of the intercostal artery. Although Fig. 6 illustrates that regional variation in stress fiber expression also exists in this area of the vessel, in general the proportion of stress fiber-positive cells in both normotensive and hypertensive animals appeared to increase as one approached the inflow tract of the intercostal artery.

STRESS FIBERS IN VENOUS ENDOTHELIUM: Stress fibers were also present in venous endothelium (inferior vena cava) of both Wistar-Kyoto and the spontaneously hypertensive rats. As illustrated in Fig. 8, these stress fibers were oriented parallel to the long axis of the vessel. Regional differences in stress fiber expression also were observed. Stress fiber expression in venous endothelium depended on location. For example, a substantial number of cells with stress fibers could be found in the infrarenal portion of the inferior vena cava, whereas the thoracic portion (just above the diaphragm) of the same vessel contained only a few endothelial cells with stress fibers.

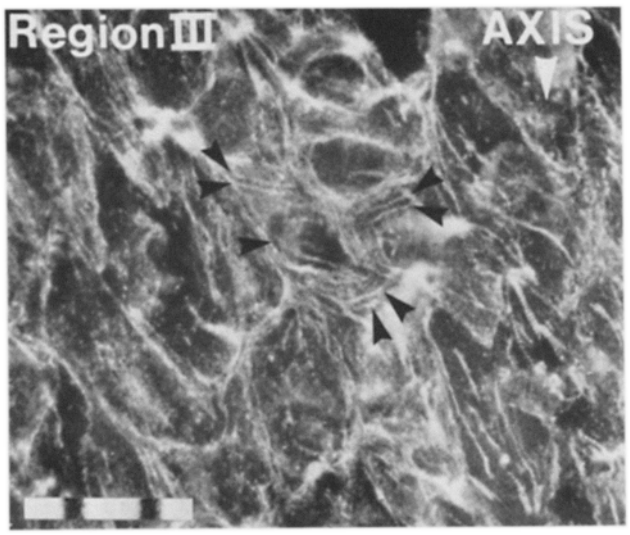


FIGURE 7 Enlargement of region III of Fig. 6. Several cells in the middle of the field do not exhibit the typical ellipsoid shape seen in other regions. Several stress fibers (arrowheads) within certain of these cells are oriented perpendicular to the long axis of the vessel (white arrowhead). Micrometer division, 10 μm.

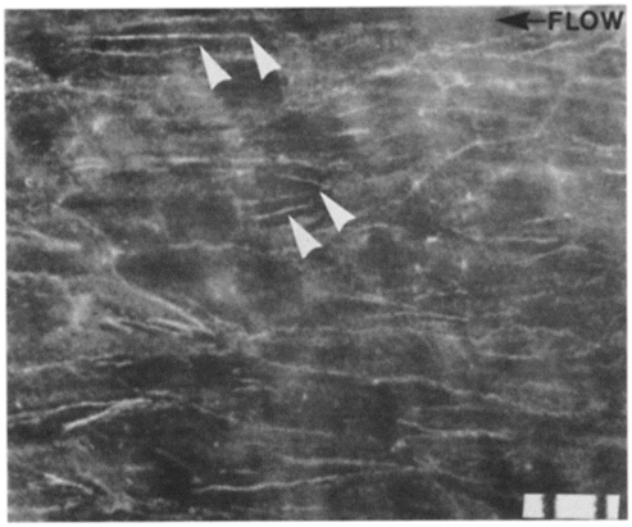


FIGURE 8 Anti-myosin staining of the inferior vena cava (infrarenal portion) of a male spontaneously hypertensive rat. Several prominent, axially oriented stress fibers are visible within these cells (arrowheads). Micrometer division, 10 μm.

## DISCUSSION

The observations presented in this report indicate that actin and myosin can be organized into stress fibers in vascular endothelial cells in situ. These stress fibers are most prevalent in aortic endothelium, where they are oriented parallel to the vessel axis. Stress fibers tend to be localized in the proximal portion of the cell. The number of endothelial cells with stress fibers varies from region to region within any given aortic segment. There also appear to be major differences in the cytoskeletal organization of the endothelial lining of the aorta compared with that of the large veins (mesenteric or inferior vena cava). Quantitative studies to determine the proportion of aortic endothelial cells with stress fibers in normotensive Wistar-Kyoto and spontaneously hypertensive rats showed that stress fiber expression is greater in the latter strain. There also are significant differences in stress fiber expression between male and female animals in either strain. These observations indicate that the expression of stress fibers within the vascular endothelium may be under the influence of several factors.

Although the existence of "actomyosin" in the vascular endothelium of various mammalian tissues has been recognized since the early observations of Becker and co-workers (1, 2), their structure and function have not been clearly demonstrated. It has been suggested that the prominent microfilament bundles observed in aortic endothelium of hypertensive rats may play a role in cell contractility and could be involved in the altered vessel wall permeability observed in these animals (17). Examples of endothelial cell contractility have been reported in microvascular and arterial endothelia in response to various vasoactive substances and inflammatory mediators (for a review, see reference 20), and stress

fibers in certain cultured cell types have been shown to be contractile (22). However, a direct link between endothelial cell contractility and in situ stress fibers remains to be established.

In tissue-cultured cells, stress fibers have been implicated in cell spreading (see for example, reference 38) and migration (4). It has been suggested that localized endothelial cell spreading and migration in vivo play a role in maintaining an intact intimal lining in the face of physiologic or pathologic endothelial cell loss (35, 36). Recent studies do not, however, support their active involvement in cell locomotion (21, 26). It also is unlikely that these processes can account for the relatively high frequency and widespread distribution of endothelial cells with stress fibers observed in our study.

The differences in the occurrence of endothelial cells with stress fibers in different blood vessels of the same animal strongly implicate local, rather than systemic, modifying factors. The relative sparsity of these structures in large veins suggests that blood pressure may be an important factor influencing their expression. However, the observed variations in stress fiber expression within a given aortic segment (see Fig. 6), which presumably are all exposed to the same intraluminal pressure, argues against this hypothesis. Another possible factor influencing stress fiber expression is cell shape. Regional variations in endothelial cell shape have been well documented (33, 37). Aortic endothelial cells in the regions examined by us tended to be ellipsoid and oriented parallel to the vessel axis (Figs. 3–7). However, as is clearly seen in Figs. 6 and 7, ellipsoid cell shape is neither a necessary nor sufficient condition for stress fiber expression in these cells.

Our quantitative studies revealed a significantly greater proportion of stress fiber-positive endothelial cells in the thoracic aorta of spontaneously hypertensive rats than in that of sex- and age-matched normotensive Wistar-Kyoto rats. Since the Okamoto-Aoki spontaneously hypertensive strain was derived by selective inbreeding of Wistar-Kyoto rats with abnormally high blood pressures, differences in the genetic make-up of the two strains are minimal (13, 28, 39, 45). Thus, the increased frequency of endothelial stress fiber expression in the hypertensive strain may be related to some factor(s) associated with elevated blood pressure.

Studies of the mechanism of hypertension in spontaneously hypertensive rats at this age (8 wk) have demonstrated that their elevated blood pressure is primarily due to a hyperkinetic circulation (30). These animals have significantly higher heart rate, cardiac output, maximum aortic flow acceleration, and peak flow velocity than age- and sex-matched normotensive controls. In light of these data, one of the hemodynamic forces that might be expected to be elevated in hypertensive rats is fluid shear stress (the frictional force created by blood flowing along the endothelial surface [8, 9]).

Exposure of vascular endothelial cells to increased fluid shear stresses may create an increased demand on the normal cellular mechanisms involved in anchorage to substratum and maintenance of structural integrity. Thus, the greater frequency and relative hypertrophy of stress fibers in spontaneously hypertensive rats may be an adaptive response of the endothelial cell cytoskeleton. That stress fibers play a role in cell adhesion was strongly suggested by studies of tissue-cultured cells in which interference-reflection and immunofluorescence microscopy were used (41). In addition, stress fibers observed in situ in fish scale scleroblasts have been postulated to be involved in the anchorage of these cells (6). However, the apparent absence of well defined stress fibers in many aortic and venous cells (see, for example, Fig. 6) clearly indicates that this cytoskeletal specialization is not required for endothelial cell attachment to the basement membrane. In addition to their possible role in cellular adhesion, it is not unreasonable to think that stress fibers also aid the endothelial cell in coping with internal mechanical stresses generated by external (i.e., cell-surface) frictional forces.

The variable distribution of endothelial cells with stress fibers in both arterial and venous vessels may be related to local variations in hemodynamic forces. For example, it has been shown that local patterns of wall shear stress can vary considerably in different regions of a large artery (27). Although the normal shear stresses in major human arteries typically range from 2 to 20 dynes/cm$^2$, such forces can be locally increased to 30–100 dynes/cm$^2$ near arterial branches and in regions of sharp wall curvature (8). In fact, endothelial cells with stress fibers do appear to be concentrated near the ostia of the intercostal arteries. While local variations in fluid shear stress may influence stress fiber expression, it is conceivable that regional variations in cytoskeletal organization may reflect localized structural differences in the vessel wall (e.g., organization of extracellular matrix). The reason for the observed sex-related differences in stress fiber expression is unclear, but they may be related to hemodynamic differences between male and female rats (e.g., male rats have higher blood pressure in both the Wistar-Kyoto and spontaneously hypertensive strains [28, 45]).

It has been demonstrated that alterations in blood flow patterns within the aorta alter endothelial cell shape (14, 33, 37); however, direct correlations between endothelial cytoskeletal organization and local hemodynamic forces are difficult to make in situ. Recent studies in our laboratories using an in vitro model system (5, 9, 10) indicate that application of physiological levels of fluid shear stress can change the shape of bovine aortic endothelial cells from polygonal to ellipsoid (9) and induce a reorientation of stress fibers within these cells (42). This reorientation consists of the coaxial alignment of stress fibers with the major axis of the cell, which becomes aligned with the direction of the applied fluid shear stress. These in vitro observations demonstrate that endothelial cell structure is sensitive to externally applied mechanical forces. Furthermore, they provide a basis for our hypothesis that stress fibers in vascular endothelium in situ are responsive to fluid shear stresses arising from normal and altered patterns of blood flow.

In conclusion, the data presented in this report clearly document the presence of stress fibers in vascular endothelium in situ and indicate that several factors influence their expression in this tissue. Anatomical (arterial, venous) and regional variations observed within the same animal suggest that endothelial cytoskeletal organization is responsive to local environmental factors. The marked difference in the number of cells containing stress fibers in aortic endothelia of normotensive compared with hypertensive rats suggests that stress fibers are influenced by hemodynamic forces. Expression of these structures may be related to the cell's need for greater adhesive capability and structural integrity in the face of elevated hemodynamic forces. The biochemical and biophysical mechanism regulating endothelial stress fiber expression and the possible role(s) of this cytoskeletal specialization in vascular cell biology and pathophysiology warrant further study.

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