Morphologic Alteration of Cultured Arterial Smooth Muscle Cells by Cyclic Stretching

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Cyclic stretching of smooth muscle cells in culture resulted in a two- to fivefold increase in protein and collagen synthesis. The same in vitro system was utilized to relate changes in smooth muscle cell morphology to mechanical stress. Smooth muscle cells, grown in culture from rabbit aorta explants, were transferred to purified elastic membranes derived from bovine aorta. The membranes were either subjected to stretching and relaxation 52 times per minute or stretched and held stationary for 8, 48, or 56 hr. Profiles of rough endoplasmic reticulum (RER) were counted and myofilament content estimated from electron micrographs of 100 cells for each experiment. Cells from cyclically stretched preparations were compared with stationary cells derived from the same subculture. Myofilaments were largely replaced by RER in cyclically stretched cells and there was a reciprocal relationship between RER and myofilament content in individual cells. In cells from stationary preparations, myofilament content also diminished with time but RER profiles were few. At 56 hr, RER profiles numbered 16.7 ± 1.7 in stretched cells compared with 3.6 ± 1.3 in stationary cells (P < 0.05). Cyclically stretched cells formed numerous intercellular contacts and showed little evidence of cytoplasmic degradation while stationary cells showed few contacts and contained numerous cytosomes and lamellar bodies. The results suggest that cyclic stretching resulted in the formation of RER or the preservation of myofilaments and that immobility resulted in the disappearance of myofilaments and cytoplasmic degradation. We conclude that cyclic stretching of arterial cells in culture under a variety of mechanical and metabolic conditions may reveal relationships among excessive mechanical stress, hormonal status, and metabolic environment in the modification of cell structure and matrix elaboration. Such studies could help to identify factors involved in the formation of arterial dysplasias.

Arterial fibrodysplasia is a pathologic entity of undetermined etiology. It consists of abnormalities of the artery wall characterized by altered morphology and disproportion and/or disarray of its usual cellular and matrix components. Mural ischemia, mechanical stress, and hormonal effects have been proposed as factors in the pathogenesis of this disorder [5, 7, 12, 13]. A role for ischemia was supported by recent experimental findings that occlusion of vasa vasorum by thrombin gel resulted in medial fibrosis [19] and that hypoxia induced morphologic alterations in cultured smooth muscle cells [11]. There has been no direct evidence that reaction of the media to physical torsion or excessive tension is a contributing factor, although this hypothesis is attractive in view of the focal nature of the morphologic changes in many instances [20]. A system for investigating the effects of mechanical stress on cells in culture has been developed by Leung et al. [8]. Utilizing this apparatus, they found that cyclic stretching markedly increased the synthesis of collagen and certain acid mucopolysaccharides by rabbit aortic smooth muscle cells but had no effect on cell proliferation [8, 9]. The purpose of the present report is to document the changes in cell morphology which correspond to the
demonstrated biosynthetic effects of cyclic stretching on arterial smooth muscle cells in culture. The findings indicate that this may be a useful in vitro model for isolating and probing the interaction between specific modes of mechanical stress and other metabolic conditioning factors in determining the structure and function of arterial smooth muscle cells.

MATERIALS AND METHODS

Methods for cell preparation, a description of the apparatus, and the procedure used to subject arterial smooth muscle cells to cyclic stretching in culture have been presented in detail elsewhere [9]. In brief, cells were grown from explants of rabbit aortic media and subcultured in modified Eagle's medium supplemented with 10% fetal calf serum. The initial explants were grown to confluence in 2–3 weeks. Elastin membranes served as the mechanical substrate for the cells. These were produced by slicing samples of bovine aorta in a cryostat and autoclaving the resulting slices to remove cells and collagen. The purified elastin membranes were mounted on Teflon frames designed to permit cyclic stretching and relaxation or to hold them stationary at any desired length. The frames with their membranes were mounted in individual culture dishes and the entire assembly sterilized by autoclaving. Smooth muscle cells (approximately $2 \times 10^6$) were then plated onto the membranes and the dishes were transferred to an incubator modified to permit the mounting frames to be connected by a train of hinged connecting rods to a motor outside the incubator. The incubator used for the preparatory cultures, and the modified incubator in which the stretching experiments were conducted, were gassed with 10% CO$_2$, 90% air, and maintained at 37°C. For each of the experiments which form the basis of this report, cells from the same subculture were transferred to two membranes. After 5 days of further growth, the dishes were transferred to the modified incubator.

One membrane was subjected to stretching to 110% of original length and relaxation to original length at the rate of 52 cycles per minute, while the other was elongated to 110% of its original length and remained stationary. Experiments were carried out for 8, 48, and 56 hr. Two trials were performed at each time.

![FIG. 1. Electron micrograph of a typical rabbit aortic smooth muscle cell following cyclic stretching on a purified bovine elastin membrane in culture for 56 hr. Only peripheral myofilaments (mf) remain. The cytoplasm is occupied predominantly by rough endoplasmic reticulum (er). Numerous cytoplasmic processes are present. Some of these processes interdigitate with those of the adjacent cell (arrows). Basal lamina (bl) is prominent. There is little evidence of organelle degradation. ×9600.](image-url)
interval. At the conclusion of each experiment, samples of the membranes were fixed by immersion in 3% phosphate-buffered glutaraldehyde at pH 7.4 for 2 hr. Care was taken to remove and fix samples with the membranes in their maximally stretched positions. This was accomplished by placing two metal ring washers, one above and one below the membrane, clamping the washers together with screws, and cutting out a circle of membrane at the outside edge of the washers. A radial marking on the upper washer was oriented so as to indicate the direction of stretch. The tissue sample within the washer center space was then cut into blocks, postfixed in 1% OsO₄ for 1 hr, followed by ethanol dehydration, Epon embedding, thin-sectioning, and staining for transmission electron microscopy (TEM).

All of the cells present on sections from each of three randomly chosen blocks from each preparation were examined. Cell profiles were then photographed for assessment of cytoplasmic components. The criterion for eligibility for quantitative study was that the cell profile show an uninterrupted cell membrane and a cross-sectional area at least two-thirds as large as the largest cell profile found for each experiment. The first 100 cell profiles to fulfill these criteria were photographed and prints prepared at identical magnifications. Rough endoplasmic reticulum (RER) was quantitated by counting the RER profiles and semiquantitative estimates of myofilament content were made by sorting the photographs repeatedly and assigning grades of 0 to 3+ according to increasing prominence of the

Fig. 2. Electron micrograph of a rabbit aortic smooth muscle cell after 48 hr of cyclic stretching in culture. Cytoplasmic processes (cp) are numerous. Profiles of rough endoplasmic reticulum (er) were less abundant at 48 hr than at 56 hr. Residual myofilaments (mf) are found at the periphery of the cell. These were most prominent in the cyclically stretched cells which contained the fewest profiles of endoplasmic reticulum. Mitochondria (m), vacuole (v). X20,000.
myofilaments in the cell profiles. Cells from cyclically stretched preparations were compared with cells grown on stationary membranes derived from the same subculture.

RESULTS

Characteristic features of smooth muscle cell morphology, i.e., myofilaments, basal lamina, dense bodies, and micropinocytotic vesicles, became less distinct with time in culture in all of the experiments but differences between cyclically stretched and stationary preparations were apparent at all of the time intervals. Smooth muscle cells subjected to cyclic stretching (Figs. 1–3) were usually elongated, with their long axes oriented mainly in the direction of stretching. Polar cytoplasmic processes in contact with, or interdigitating with, adjacent cells were numerous. Ribosomes and RER were abundant and were most prominent in the cells which were subjected to prolonged cyclic stretching (Fig. 1). Strips of myofilaments were found in most of the cells and basal lamina was often extensive. Cells on stationary membranes (Figs. 4 and 5) showed only occasional cell interdigitations or junctions and lacked any specific pattern of cell orientation. Profiles of basal lamina were frequently interrupted, attenuated, or absent. RER, mitochondria, ribosomes, and Golgi complexes were sparse. Myofilaments were greatly reduced, particularly in central portions of the cell bodies, but there was no consistent change with time during the experimental period. Unlike cyclically stretched cells, stationary cells contained numerous myelin figures, cytosomes, and laminar bodies as well as vacuoles.

Quantitation revealed that RER profiles were five times more abundant in stretched than in nonstretched cells (Table 1) and appeared to increase with duration of cyclic stretching. Cells that were stretched for 56 hr had 25% more rough endoplasmic reticulum than cells stretched for 8 hr. In addition, di-

![Fig. 3. Electron micrograph of a smooth muscle cell following 8 hr of cycling stretching. Lipid droplets (l) and cytosomes (cs) were seen in both stretched and stationary preparations, but were much more abundant in stationary preparations. Rough endoplasmic reticulum (er), mitochondria (m), and cytoplasmic processes are evident (cp). ×8000.](image-url)
FIG. 4. Electron micrograph of a smooth muscle cell following 8 hr of culture on a stationary elastin membrane. Myofilaments (mf) are evident as well as peripheral dense bodies (db). Some rough endoplasmic reticulum (er) and a few ribosomes (r) are also present as well as occasional cytosomes (cs). Mitochondria (m). ×10,000.

FIG. 5. Electron micrograph of a typical rabbit aortic smooth muscle cell after 56 hr of culture as a stationary preparation. Only remnants of myofilaments (mf) remain but rough endoplasmic reticulum is also sparse. Cytoplasmic processes are few. Cytosomes (cs) and lamellar bodies (lb) are numerous. ×10,000.
TABLE I

<table>
<thead>
<tr>
<th>Duration in culture (hr)</th>
<th>RER profiles/cell section&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stretched</td>
<td>Nonstretched</td>
</tr>
<tr>
<td>8</td>
<td>11.8 ± 1.2</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>48</td>
<td>14.2 ± 1.4</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>56</td>
<td>16.7 ± 1.7</td>
<td>3.6 ± 1.3</td>
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*Note.* Profiles of rough endoplasmic reticulum in cyclically stretched and in stationary cell smooth muscle cell cultures. Each determination is based on the examination of 100 cells.

*a* Mean ± SD for 100 cells for each determination.

Cytosomes and myelin figures were especially prominent in the stationary preparations. In contrast, the cytoplasm of smooth muscle cells subjected to cyclic stretching contained abundant rough endoplasmic reticulum and dilated cisternae. RER profiles were 4–6 times more abundant in cyclically stretched cells depending on duration of stretching. This observation is consonant with the two- to fivefold increase in protein and collagen synthesis by cyclically stretched aortic smooth muscle cells reported by Leung et al. [8, 9] using the same system. This close correspondence indicates that quantitative correlations can be established among cell morphology, biosynthesis of matrix fibers, and mechanical stimuli for smooth muscle cells under these experimental conditions.

Worthy of note is the finding that the cyclically stretched cells showed evidence of modulation from a predominantly contractile to a predominantly biosynthetic mode with minimal evidence of organelle degradation and a reciprocal relationship between myofilament and RER content. The stationary smooth muscle cells, not subjected to cyclic changes in tensile stress, showed both a marked reduction in myofilaments and relatively little increase in rough endoplasmic reticulum. The absence of tensile stimulation apparently reduced cell function to basal maintenance levels in the stationary preparations and favored the involutional and degenerative changes usually noted in culture, while cyclic stretching induced increased matrix biosynthesis or maintained a complement of myofilaments. Such findings indicate that cultured arterial smooth muscle cells may be capable of a range of responses to tensile stimuli. One should be able to find the level of stretch amplitude and frequency, in vitro, which maintains the cell in a predominantly contractile or a predominantly biosynthetic mode for extended periods.

The ability of an arterial wall to withstand the tensile forces associated with changes in size during growth and with increases in blood pressure, and to recover from mechanical injury [21], requires cell proliferation as well as modifications in matrix composition and architecture of the media. The mechanisms by which this adaptation is accomplished are not clear. There is, however, evidence that the rate of connective tissue production by smooth muscle cell during normal growth [10] and in response to hypertension [22] is closely related to the magnitude of tensile stress imposed on...
the vessel wall. Under abnormal metabolic conditions and in response to chronic mechanical trauma, arterial smooth muscle cells may respond without maintaining normal proportions of matrix or normal architecture depending on size and location of the vessel. Following physical injury, for example, smooth muscle cells of the coronary artery tend to synthesize mainly collagen, while those of the thoracic aorta increase elastin production predominantly [17]. Hyperlipidemia has been shown to interfere with healing of the media following an acute transmural injury [2]. Modification of, or interference with, normal biosynthetic [15, 16] regulatory and reparative [14] mechanisms by excessive and prolonged mechanical deformations, altered hormonal status, and/or hypoxia may contribute to the development of artery wall dysplasias. Ross and Klebanoff have reported that estrogen enhances protein synthesis by uterine smooth muscle cells [18]. Similar effects of hormones on vessel walls are suggested by the findings of Bo et al. [1]. Reduction in nutrient supply to the vessel wall has been shown to increase accumulation of collagen and certain mucopolysaccharides with concomitant morphologic alteration of the smooth muscle cells [12, 13, 19]. Arterial changes in pulmonary hypertension [6], as well as intimal fibrocellular hyperplasia in vein grafts [3, 4], are probably examples of reactions to prolonged, abnormally increased medial stresses. Age may also play a role. Although caution must be
exercised in extrapolating the results of mechanical stretching of smooth muscle cells in vitro to mural dysplasia in vivo, our system has the advantage of permitting the independent evaluation of many of the variables which may modulate smooth muscle response. Results of such experiments could help to establish the pathogenic significance of hormonal, nutritional, and mechanical factors in the development of the morphological features associated with arterial dysplasias.

REFERENCES