Mechanical stresses keep endothelial cells healthy: beneficial effects of a physiological level of cyclic stretch on endothelial barrier function

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The cells of our bodies live in a sea of chemical and mechanical stimuli. A cell can be stimulated by a variety of molecules dissolved in body fluids as well as by the extracellular matrix and molecules expressed on the surface of other cells. These molecules (or ligands) are recognized by specific receptors, most of which are transmembrane proteins. Ligand binding activates receptor molecules, initiating intracellular signaling. The set of receptors expressed by a cell determines whether or not the cell responds to a specific ligand. In addition to the chemical milieu, cells are also exposed to mechanical forces, including frictional forces, stretch, and pressure, and some cells are known to respond to these mechanical forces. Bone remodeling and exercise-induced muscle fiber thickening are examples of the body’s responses to mechanical stress. Vascular endothelial and smooth muscle cells have been shown to respond to mechanical stimuli. Both in vitro and in vivo studies have shown that significant changes in morphology, physiology, biochemistry, and gene expression occur in endothelial cells exposed to fluid shear stress and cyclic stretches. This is presumably the result of activation of various signaling cascades by the mechanical stresses. Experiments studying the mechanoresponses of these cells typically use force levels in the range of 1–50 dyn/cm². These levels of fluid shear stress are considered to be physiological (at rest) for human arterial endothelial cells but are quite small compared with the magnitude of forces the body must experience during daily activities. For example, one atmospheric pressure is ~10⁶ dyn/cm². The magnitude of forces the body is exposed to must be substantially higher than 50 dyn/cm² when a person is engaged in sporting activities and hard physical labor. How do cells process such strong mechanical stimuli (since endothelial cells are affected by much smaller levels of forces)? Because the body does not appear to go into signaling chaos during strong physical activity, either most cells simply ignore mechanical stimuli (i.e., have no mechanism for sensing mechanical stress) or these forces are not transmitted to cells. For example, the bulk of mechanical forces may be absorbed by noncellular elements of the body so that cells are insulated from these forces.

Breathing causes lung tissue to stretch cyclically. Thus endothelial cells of lung vessels are also cyclically stretched. Because there is no easy way to estimate how much force is required to stretch a cell by a known amount, the extent of stretch is expressed by the percent the cell is elongated. The amount of lung tissue stretch under various breathing conditions is difficult to determine, but a linear stretch of 5% is thought to be a reasonable estimate during normal breathing. Ventilator-induced lung injury is characterized by increased vascular leakiness and inflammatory processes. During mechanical ventilation at high tidal volumes, endothelial cells could be stretched as much as 17–22%. This level of stretch might damage cells, causing leakiness of the endothelium. In the study by Birukov et al., one of the current articles in focus (Ref. 1a, see p. L785 in this issue), the authors report how excessive cyclic stretch might contribute to the observed failure of endothelial barrier function. These investigators cultured a confluent monolayer of human pulmonary arterial endothelial cells on a flexible membrane, which was then cyclically stretched by 5% (physiological level) or 18% (high tidal volume stretch) for 0–48 h. Interestingly, no apparent damage to the endothelial monolayer occurred even after it was cyclically stretched 18% for 48 h, suggesting that ventilator-induced lung injury cannot be explained by the most simplistic idea of stretch-induced mechanical damage. Their study shows that cyclic stretch of different magnitudes affects differentially the responsiveness of endothelial cells to thrombin, an edemagenic substance. This effect appears to be caused by some phenotypic changes that occur during the 2-day exposure to cyclic stretch.

The most remarkable observation reported is from the experiments in which they analyzed transendothelial monolayer electrical resistance, a measure of the barrier function, after cells were preconditioned by cyclic stretch for 2 days. To do these experiments, the authors needed to replate cells on gold electrodes that were used to measure electrical cell-substrate impedance. Preconditioned cells were harvested by trypsinization, plated into culture chambers equipped with gold electrodes, and allowed to spread for 16 h, and then the electrical resistance of the monolayers was measured. Without thrombin stimulation, the endothelial cells preconditioned with a 5 or 18% cyclic stretch and those not preconditioned (static) exhibited similar levels of transmonolayer electrical resistance, indicating that mechanical stimuli did not affect the basal barrier function. When thrombin was added to these cultures, electrical resistance decreased rapidly as expected, but the way in which these differently preconditioned cells
responded to thrombin was extraordinary. The most severe loss of electrical resistance occurred in the monolayer of cells treated with 18% stretch, and the least affected were the cells preconditioned with the physiological level of cyclic stretch, not the static control. The cells preconditioned with 5% stretch were not only least affected but also showed the fastest recovery from the thrombin-induced damage in barrier function. The effect of cyclic stretch on the recovery from thrombin assault was very dramatic. At the end of 4 h of recovery, electrical resistance returned to almost 100%, ~30%, and ~20% of the prethrombin treatment level in cells preconditioned with 5% stretch, not preconditioned (static), and preconditioned with 18% stretch, respectively. These data appear to indicate that physiological levels of cyclic stretch exert beneficial effects on endothelial cells. This is reminiscent of the fact that physiological levels of laminar fluid shear stress are atheroprotective (8). These studies suggest an interesting paradigm that ordinary levels of mechanical stress exert beneficial effects on endothelial cells.

Another remarkable observation is that this beneficial effect (and also the negative effect of being stretched 18%) was not lost even after cells were cultured under static conditions for 16 h. This is contrary to the many types of flow responses by these cells that are transient or rapidly reversible. The results reported in the article clearly indicate that endothelial cells “remember” the mechanical environment to which they were exposed and suggest certain phenotypic changes occurred in them. cDNA microarray analyses revealed upregulation of several genes that might be involved in endothelial barrier function. It is interesting to note that certain genes such as HMG-CoA-synthase, cyclooxygenase-2, and connexin40 were activated in cells exposed to 18%, but not 5%, stretch for 48 h. Although at present, the biological significance of the increase is unknown, one wonders whether the upregulation of these genes relates to the deleterious effect of overstretching. Cadherin-13 expression was substantially increased in the 5% stretched cells. Although this cadherin isoform does not appear to be localized to the adherens junction, it does localize to the interendothelial cell junction. It is possible that highly increased expression of this cell adhesion protein helps seal the gaps in the endothelial monolayer created by thrombin treatment. It might be interesting to see whether preconditioning cells with 5% stretch or overexpressing cadherin-13 reduces the effects of stretching 18%. The study does not describe genes that were downregulated in the stretched cells. The observed stretch effects could be due to reduced expression of certain genes.

Among the upregulated genes was ras-related rho. Rho and rho kinase play central roles in the regulation of stress fibers, which are actomyosin-based contractile structures. Their contraction is caused by phosphorylation of myosin regulatory light chain, which appears to be regulated by both myosin light chain kinase activated by Ca^{2+}/calmodulin as well as Rho kinase (5). Endothelial cells are one of the few cell types that express stress fibers in both cultured and in situ cells (10), and both cyclic stretch and thrombin treatment increase stress fiber expression in endothelial cells. Garcia and colleagues (4) have been investigating the role of actomyosin contraction in thrombin-induced vascular dysfunction and found that thrombin treatment induced myosin light chain phosphorylation, causing endothelial cells to contract. It is thought that the contraction of an increased number of stress fibers creates tears within the endothelial cell monolayer, causing barrier dysfunction. Consistent with this idea, Birukov et al. (1a) clearly demonstrate that inhibition of myosin light chain phosphorylation abolishes thrombin-induced gap formation in endothelial monolayers. These results are also consistent with recent studies by others (3, 9).

Although further studies may be necessary, this study suggests that cyclic stretch per se is not the direct cause of ventilation-induced lung injury. However, it clearly indicates that cyclic stretch above the normal physiological levels makes cells more susceptible to edemagenic conditions. Studies by these and other investigators demonstrate the involvement of Rho in the process of thrombin-induced barrier dysfunction (1a, 2, 9). However, the precise molecular mechanism by which Rho is activated by thrombin or cyclic stretch is not known at present. Elucidation of this mechanism is a matter of high priority for understanding the pathophysiology of vascular barrier dysfunction. Also unknown, despite significant progress made in recent years, is the mechanism of mechanosensing by endothelial cells. Are there specific sensor molecules that transduce mechanical stimuli into chemical signals? If there are, are they activated by both fluid shear stress and stretch since many of the endothelial cell responses are common to both shear stress and stretch? We have recently reported that platelet/endothelial cell adhesion molecule-1 (PECAM-1) is a mechanoresponsive molecule that can be tyrosine phosphorylated by fluid shear stress, osmotic changes, and the direct application of a tugging force (7). Our recent studies indicate that cyclic stretching also causes PECAM-1 tyrosine phosphorylation (Shiraki H, unpublished observation). Thus this cell adhesion molecule may be a general mechanosensor for endothelial cells. The spatial (6) and temporal (1) gradients in shear stress, not the absolute level of shear stress, were found to be the key factor for certain endothelial cell responses. Thus it is possible that endothelial cells respond also to the spatial and/or temporal gradients in stretch gradient. Another important parameter for cyclic stretching is frequency. The present experiments were done using only one frequency. Further experiments along these lines might provide additional insight into the mechanism of stretch sensing and response by endothelial cells.

REFERENCES


